APR - 2 1934

Vol. 61 APRIL, 1934 No. 4

143

Bulletin

of the

Torrey Botanical Club

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Published for the Club monthly except

July, August, and September

by

GEORGE BANTA PUBLISHING COMPANY, MENASHA, WISCONSIN

Subscription price $5.00 per year

Entered as Second Class Matter at the Post Office at Menasha, Wisconsin
Accepted for mailing at special rate of postage provided for in the act of February 28, 1925, embodied in paragraph 4, section 412, P. L. and R., authorized July 23, 1928.

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Bulletin. Established 1870. Monthly, except during July, August, and September. Price, $6.00 a year; single numbers 75 cents. Of former volumes, only 24-56 can be supplied separately; certain numbers of other volumes are available, but the entire stock of some numbers has been reserved for the completion of sets. Manuscripts intended for publication in the BULLETIN should be addressed to Prof. M. A. Chrysler, Department of Botany, Rutgers University, New Brunswick, N.J. The editors may accept papers up to 20 pages in length, with illustrations and tabular matter generally not exceeding twenty per cent of that amount. Longer and more copiously illustrated papers, however, may be published if the excess pages, illustrations, and tabular matter are paid for by the contributors.

Torreya. Bimonthly, established 1901. Price, $1.00 a year. Manuscripts intended for publication in TORREYA should be addressed to Mr. George T. Hastings, Editor, 2587 Sedgwick Ave., New York, N.Y.

Memoirs. Occasional, established 1889.

Preliminary Catalogue of Anthophyta and Pteridophyta within 100 miles of New York City, 1888. Price, $1.00.

Reprints. Contributors of articles accepted for publication in the BULLETIN should order reprints, if desired, when they return galley proofs to the editor. They may be ordered through the editor from the George Banta Publishing Company, the contributor paying for all his reprints. A schedule of rates is sent with galley proof.

A cytological study of the early infection stages of the black rot of cabbage

DOROTHY MEIER

(WITH PLATES 9–12)

INTRODUCTION

The fact that bacteria are the cause of certain plant diseases has been known since the latter part of the nineteenth century. The first extensive pathological work with the cabbage dates back to 1893 when Pammell upon examining turnips, *Brassica campestre*, with yellow, withered leaves and blackened veins, suspected the presence of bacteria and succeeded in isolating motile, yellow organisms from the plants. He termed this organism *Bacterium campestre*.

The organism was found to be causing great financial losses among cabbage and turnip growers in the middle west soon after Pammell's discovery, and the interest of phytopathologists of the state and national departments of agriculture was aroused. Detailed studies of the characteristics of the organism, the mode of infection, and its course through the host tissue were made as early as 1896 by Smith and Harding.

The size of the bacilli vary when isolated from different parts of the plant. When first introduced into the plant from agar cultures it varies in size from 5–7μ to 4–5μ. When crowded in the vessels of the host it often loses its motility and becomes smaller and resembles a micrococcus.

It has been found that the organism enters the plant by means of the specialized water pores at the teeth of the leaves or by means of insect bites. The affected plants show blackened veins and ultimately dry, yellow leaves, and the path of the parasite can be traced by the blackened vascular strands in the leaves, petioles, stems, and roots. The disease is essentially a dry rot, but in the field it is often followed by invasion of secondary organisms which cause a wet rot with strong odors of decay.

The more recent investigations of bacterial diseases of plants have been primarily concerned with the problems of the manner and mechanism by which the organisms travel in the host tissue, their course in the plant, host and parasite relationship, and the manner in which they gain entrance to the cells. In general four different hypotheses have been offered as to how the organisms advance in the host tissues: growth by reproduction of the bacteria in the direction of the food supply; motility of the individual organisms; a gelatinous secretion or a zoogloea which pushes its way through the intercellular spaces of the host and carries the bacteria with

[THE BULLETIN FOR MARCH (61: 103–172) WAS ISSUED MARCH 5, 1934.]

it; and by mechanical means through which the bacteria are passively carried through the tissue.

As to the relation between parasite and host tissue it has been shown by various investigations that the action of different types of bacteria is not always similar. Certain organisms are normally found within cells and others are intercellular parasites. For example, *Bacillus amylovorus* that causes fire blight of pear is reported by Nixon to pass between the cortical cells of the twig. *Bacillus radicicola* passes through the interior of the cells of the roots of clover and brings about hyperplasia of cells. *Bacterium tumefaciens* is an organism that is reported to be intercellular but it also brings about abnormal production of cells. *Bacterium campestre* is an intercellular parasite until it gains entrance to the tracheids. From then on the life of the organism is spent within the water vessels, and the death of the tissue is due to thrombosis, or the shutting off of water and mineral supply from the uninfected vessels below.

Various theories have been advanced as to the manner in which organisms enter cells, whether they dissolve openings in the cellulose walls; pass through pits or thin places in the walls, as suggested by Smith; whether in a minute form they are able to squeeze through spaces between molecules, as advanced by Burrill; or whether there is a filterable stage in the life history of bacteria during which they diffuse through the cell walls, as maintained by Kendall, Sherman, and Safford.

The present investigation was undertaken primarily to trace the progress of *B. campestre* from its first appearance in the pores at the teeth of the leaf to its entrance into the water vessels to determine how the bacteria enter the plant, their manner of progress through the host tissue, the effect of the bacteria on the host tissue, and to compare its method of migration with that of other bacteria causing plant diseases.

REVIEW OF LITERATURE

The literature dealing with bacterial diseases of plants in general is an extensive one and has been amply reviewed by E. F. Smith and other members of the U. S. Department of Agriculture and by other investigators of this country and Europe. I shall consider only such papers as relate to the problems of infection, progress of the bacteria in the host tissue, method of migration, and host and parasite relationship.

It is agreed by most investigators that bacteria have a destructive influence on the contents of the cells that they surround. This was proven first by Burrill (1881), one of the pioneer plant pathologists, who studied the relations of *Bacillus amylovorus* to the pear. He believed that the bacteria could enter cells, but since walls appeared unaltered until the late stages

of the disease when they turned brown, he stated that the organisms might be able to pass into cells in an ultra-microscopical condition, or perhaps in the so-called germ condition they could, amoeba-like, creep through the spaces between the molecules. He found that the bacteria caused the disappearance of starch in the cells, although the remainder of the protoplasm was apparently not affected by them.

The work of Burrill on *Bacillus amylovorus* was followed by that of Arthur (1886). He stated that bacteria first attack the starch of the cells, then the cellulose walls, until finally "the whole tissue becomes a liquid mass." He found that in the living plant the organism produces "a mucilage or gum, which is soluble in water,—with the disengagement of carbon dioxide."

In 1891 Garman first noticed a disease of cabbages where the infected plants showed black veins and yellow leaves. By observations throughout the entire season he came to the important conclusion that the organisms are able to "invade and break down the tissues of the plant" only during periods of high temperature and excessive moisture.

The isolation of the bacterium causing the black rot of cabbage was actually accomplished by Pammell (1893–1895), who, as noted before, isolated a motile, yellow organism from the turnip and called it *Bacterium campestre*. He confirmed the work of Garman when he found that the characteristic yellowing of the leaf parenchyma and the blackening of the veins is checked by the dry weather of September, and made the interesting discovery that plants infected with the organism recovered by the formation of a corky layer about the diseased portions.

With the establishment of the fact that bacteria are the causes of plant diseases and are of considerable economic importance to plant growers, more detailed studies were begun with an attempt to find how the bacteria gain entrance to the tissues, and how they travel through the different parts of the plant. It was first maintained that progress of the parasite through the host tissue occurs by multiplication of the organisms in the direction of the food supply and by the motility of individual organisms.

Erwin F. Smith made detailed studies of *B. campestre* during the years 1897–1911. He found that turnips, cabbages, and many members of the genus *Brassica* were infected by the organism through the water pores at the teeth of the leaves. He stated that the bacteria grow in the fluid extruded from the water pores and move in through these pores away from the light toward the direction of the food supply. Entrance through stomata was impossible, in his opinion, because of the waxy bloom on the surface of the leaf which prevents the surface from becoming moist. He found that

the progress of the bacteria towards the vessels is slow,—probably due to insufficient water supply or inability of the bacteria to dissolve the cell walls immediately and thus pass from the water pores to the veins. He stated that the juices of the parenchyma are not so well adapted to the needs of the organism as the fluid inside the vessels. Lack of sufficient aeration was the cause, in his opinion, of the slow sidewise movement of the organism in the stem, which thus prevents destruction of the plant.

Smith believed that the bacteria probably enter the vessels by pits or thin places in the wall.

While Smith was carrying on his experiments with the black rot organism Russell was likewise making a study of the disease, and in his paper published in 1897 stated that the bacteria develop in drops at the teeth of the leaves and by their motility work their way in through the water pores.

Hecke (1902) is in agreement with Smith when he states that the movement of *B. campestre* in the tissues is due to growth, not self motility.

Jones (1909) claims that "aside from the moisture content, susceptibility to infection is largely, if not wholly, dependent on the nature of the middle lamella." Forty-five strains of bacteria causing black rot in species of *Brassica* were found to secrete an enzyme that dissolves the middle lamella. Complete solution of the cellulose and diastatic action were found to be lacking.

The importance of moisture mentioned by previous writers was also stressed in a short account of the life history of the black rot organism by Coulter, Barnes, and Cowles, 1910, who attribute the entrance of the bacteria into the tissues to mechanical means. Bacteria that are lying on the surface of leaves come in contact with the fluid of guttation given off at the hydathodes when evaporation is checked. This fluid is resorbed when transpiration begins again in the leaf, and the bacteria by contamination of the hanging drop pass into the chamber beneath the pore. Here they develop and "kill the adjacent cells, whence they enter the xylem and work backward, killing and rotting the bundles."

Differing to some extent with the previous work of Burrill and Arthur on *B. amylovorus*, Bachman (1913) stated that the bacteria in apple tissue are surrounded by sap from the cells. This film of liquid does not precede the bacteria to any extent. She claimed that the host cells die because of loss of water, and that cell walls were ruptured by physical means or by osmotic pressure.

For a number of years investigators had attempted to stain plant tissues infected with *Bacterium tumefaciens*, the causal organism of crown gall in tomato and other plants. In 1923 Riker, working with these bac-

teria, found that a chemotactic response to expressed tomato sap makes it possible for *B. tumefaciens* to enter surface wounds. He found that the galls which develop as a result of bacterial invasion coincide in outline with the water-soaked region formed by wounding the tissue. The bacteria are distributed in the liquid of the intercellular spaces.

In recent years the majority of investigators have emphasized the gelatinous matrix or zoogloea as the means of movement of bacteria through the plant tissues. The ability of *B. amylovorus* to form this zoogloea in fluid cultures was first noted by Arthur in 1886, and Smith in 1896 showed that *B. campestre* will also form a zoogloea in fluid cultures. In 1904 Peirce stated that the root nodule bacteria enter cells by softening or dissolving the cell walls. They move or grow through the opening, multiply rapidly, and form a thread-like zoogloea which grows chemotropically through the cells toward the nuclei.

Bacterium tumefaciens is figured by Robinson and Walkden (1923) as showing zoogloeal strands that intrude along the intercellular spaces and proto-xylem strands of infected plants. This work was confirmed by Hill in 1928 and studied further by Hill, Brittingham, Gibbons and Watts in 1930. Nabelek (1930) found these organisms traveling through the tracheae and between cambium and conducting cells of the geranium. He reported that the zoogloeae dissolve the middle lamellae and that the organisms cause hypertrophy of cells and the metaplasie of cells into secondary tracheal elements at the wound surface.

Nixon (1927) found that *B. amylovorus* migrates through the tissues of water sprouts of the apple in the form of a zoogloea. Migration is intercellular in early stages. The matrix of the zoogloea is jelly-like with finely granular or homogeneous structure. The bacteria cause plasmolysis of the protoplast which leads to its collapse. Haber, in her study of *B. amylovorus* in leaf tissues of the apple in 1928, and Wahl in his work with the organism in the quince confirm the work of Nixon.

Pseudomonas pisi forms an "abundant slime" having "high absorptive powers" in the tissues of the pea, according to Skoric (1927).

Although Hill (1928) observed that motion of *B. tabacum* through the intercellular spaces of the tobacco is due to a zoogloea he stated that the zoogloea appeared to do little or no harm to the host cells.

B. vignae, according to Beach (1928) migrates through the intercellular spaces of the lima bean in zoogloeal form, and brings about disintegration of cell contents.

Ivanoff and Riker (1930) contend that purely mechanical means are influential in the migration of bacteria through the host tissues. By their studies of *B. tumefaciens* in the tomato they conclude that the mechanism

by which the bacteria and inert substances move through the intercellular spaces is not definitely understood, but they suggest that capillarity and negative pressure are important factors.

The contention that certain bacteria exist in both filterable and non-filterable states has recently been advanced by Kendall, Sherman, and Safford (1931). Kendall maintains that *B. typhosus*, *B. coli*, and *Streptococci* when grown on suitable protein medium were rendered filterable, but by suitable procedure could be regained again in non-filterable form. Sherman and Safford report the presence of filterable forms of bacteria in various sources including milk and hay. They believe that these "primitive" types are probably present in greater numbers than the familiar forms of bacteria.

The formation of zoogloeae by three organisms parasitic on the bean, *B. flaccumfaciens*, *B. medicaginis*, *B. phaseoli*, was reported by Zaumeyer (1932). The organisms were embedded in a slimy matrix with high absorptive power which enabled them to rupture cell walls and cause the disintegration of cells.

MATERIALS AND METHODS

The plants used in this study were chiefly the Danish Ball Head and Flat Dutch varieties of cabbage and Best of All cauliflower, which were grown in the greenhouse at Columbia University. At the time of inoculation they varied in size from seedlings with two or three leaves to mature plants about eight months old.

The organisms used for the inoculations were obtained from the U. S. Department of Agriculture. The pathogenicity of the bacteria and the susceptibility of the plants to the disease were previously tested by stab inoculations into healthy plants and subsequent isolation of the black rot bacilli from the blackened veins which developed. The organisms recovered from these plants were then grown for two or three days on potato agar before inoculations were made.

As reported by early investigators infection in the field takes place through the teeth of the leaves when drops of guttation water are present. Accordingly an attempt was made in this study to simulate these infection conditions in the greenhouse as nearly as possible. Plants were placed under bell jars until drops of water appeared at the teeth of the leaves and then sprayed with a heavy suspension of bacteria in distilled water. Five to ten minutes were occupied in spraying the plants in this manner. They were then covered again with the bell jars for several hours and finally exposed to the air of the greenhouse.

Inoculations were also made directly into the stems by smearing small

areas below the nodes with bacteria and pricking deeply with a needle. Controls were stabbed with a sterile needle.

In order to observe the progress of the bacteria in the tissues permanent mounts were made. Portions of the margins of the leaves approximately .5 by .5 cm. were removed and fixed immediately after inoculation and at intervals of one to 24 hours and every succeeding day for 10 days. Flemming's weak, strong, and medium, Merkel's, and Allen and Wilson's modification of Bouin's killing solutions were used for fixation. The latter solution proved to be the most satisfactory. The material was embedded in paraffin and sections were cut 7–10μ thick. The stains used were iron-alum haematoxylin and Flemming's triple. Both stains were satisfactory but the triple stain was of particular value owing to the differentiation between the host and parasite.

An effort was made to make flagella stains of the bacteria in the tissues at different time intervals, but so far this has not succeeded. Sections of the teeth of the leaves were cut parallel to the surface and these sections were mounted in water for study of the motility of the organisms. The progress of the organisms in the stem and the effect brought about on the host cells as a result of stab inoculations was watched by means of hand sections as well as by permanent mounts.

OBSERVATIONS

The progress and invasion of bacteria in most hosts are dependent to a certain degree on the compactness and continuity of the tissues through which they travel, particularly when moving in large zoogloeal strands as in the case of *Bacillus amylovorus*. It is therefore essential at the outset to give a brief histological description of the structure of a normal uninfected cabbage leaf with special reference to the intercellular spaces, subepidermal chambers, cell contacts, and continuity with the vascular system.

The surface of the epidermal cells of the leaf is covered with a thick coat of cutin which prevents water from collecting in large drops on the leaf. In longitudinal and stained sections of the cabbage leaf, the tooth or terminus of tracheids appears thicker than the adjoining regions, owing to an abundance of small epithem cells which surround the apices of the tracheids as is shown in figure 1. The epithem cells are loosely arranged in relation to each other, with many intervening intercellular spaces.

They are frequently completely free of chloroplastids or at most contain only one or two. The cytoplasm, with nucleus embedded in it, appears as a thin layer just inside the cell wall.

Immediately beneath the upper and lower epidermis are three or four

rows of chlorenchyma cells. They are loosely packed together with large intercellular spaces below the upper, but appear to be closer together above the lower epidermis of the leaf. The large water pores of the epidermis of the tooth, or hydathodes, lead into cavities which are similar in structure but larger than those beneath the stomata (fig. 1a). In fixed material the water pores are always open. The width of the pore varies from 1.5μ, as is shown in figures 3 and 4, to 6μ as in figure 1b.

The tracheids do not follow a straight path but curve in and out among the chlorenchyma and epithem cells and end with one or two tips directed toward the hydathodes (fig. 1c, 6b, and 8). The spiral thickenings of lignin are connected with thin, anastomosing strands, and the cellulose portions of their walls appear thinner than the wall of the epithem cells which surround them.

Under normal conditions the water which is not used in the metabolism of the plant evaporates from the stomata. When the plant is kept very moist, however, and evaporation is lessened by reason of the high relative humidity of the air, drops of water appear at the teeth of the leaves. During such periods of high relative humidity, when there is a continuous pathway of liquid from tracheid tips through the water pores, infection with the black rot organism normally takes place.

Description of leaves sprayed with the bacteria. Fixed and stained preparations of inoculated cabbage leaves show that the bacteria enter only at the hydathodes. Bacteria were observed variously oriented in a film of blue-stained material on the epidermis and over stomata on leaves sprayed 7 days previously, but they did not gain entrance into the leaves in these regions. The film in which they lie appears sufficiently thick and consistent to support the weight of the bacteria, and extends about 6μ above the surface of the leaf, as is shown in figure 2. This same blue-staining material is also present in stained smear preparations on slides. The substance that stains blue appears to be secreted by the bacteria as soon as they are placed in sterile distilled water.

The first visible evidence of the bacteria in the pore cavities has been found 3 hours after spraying, as is shown in figure 3. In some preparations bacteria lie in the spaces between cells that border the cavity. They are stained red and lie in a thin film of blue material. In certain regions they are scattered loosely about in the intercellular spaces without any particular orientation. Between other cells they are packed more closely together, so that it is difficult to distinguish individual organisms. The blue material in which they lie fills the intercellular spaces completely. It none the less appears less dense than the zoogloeae of *B. amylovorus* and *B. tumefaciens* figured by Nixon, Haber, Hill, Riker, and Ivanoff, and reported

to push their way between host cells with blunt advancing tips. In places the bacteria appear wedged between cells so that adjacent cells are forced apart (fig. 3a). The blue-stained material is not distinguishable in advance of the bacteria in these regions as in the case of forms with zoogloeae.

At this time it is apparent that cells with masses of bacteria about them are slightly affected. The cytoplasm appears slightly plasmolyzed, the nucleoli have disappeared from the nuclei, and nuclei and chloroplastids have collapsed in the cytoplasm against the walls. The cell contents stain less densely than formerly. With a view of bringing out more specifically the host and parasite relationship at this stage I have drawn in figure 7 cells from the epithem regions of infected and uninfected plants.

The rate of the bacteria in entering and passing through the tissue varies. In many preparations of leaves sprayed 6 days before fixing bacteria have been found widespread throughout the entire tooth and can be traced from the water pores to their entrance into the tracheids. In other preparations, on the other hand, bacteria are present in great numbers between cells but have made less progress. Figure 4 shows bacteria among cells of a leaf that was sprayed 7½ days before fixing. The organisms have not reached the tracheids, but are present in great abundance among the cells just beneath the pore cavity, filling the intercellular spaces completely. The homogeneous blue-staining material in which the bacteria lie is hard to distinguish here because of their great number. At least it is not visible in advance of the bacteria as described by Nixon, Haber, and Nabelek for *B. tumefaciens*, and Zaumeyer for *B. flaccumfaciens*, *B. medicaginis*, and *B. phaseoli*. At this time the bacteria appear definitely oriented with their long axes parallel and their ends directed toward the intercellular spaces which lead to the interior of the epithem region. The organisms are densely aggregated about the cells where the lamellae are still intact. It is difficult to distinguish between nuclei and chloroplastids in the cells that have bacteria about them. The nuclei, with no visible nucleoli, and the chloroplastids, appear collapsed in the scanty layer of lightly staining cytoplasm that surrounds these cells.

In preparations with bacteria in the intercellular spaces throughout the entire epithem region, the middle lamellae have been destroyed, and where the intercellular spaces are narrow the bacteria lie with their long axes parallel to the cell walls (fig. 5). In certain regions cells surrounded by bacteria appear to have lost their turgor and the walls have collapsed (fig. 6a). In these particular regions and also in the large intercellular spaces the bacteria are not crowded together and definitely oriented. The blue-staining homogeneous background in which they lie is much denser in the

deeper lying regions of the tooth than immediately beneath the pore cavity.

The organisms maintain their motility in the host tissue during the early infection stages. This was ascertained by examination of sections of the living leaf cut parallel to the surface. The bacteria moved in various directions with quick, darting motion made possible by the one-polar flagellum. Although motion was not in any one definite direction it was less hampered and more free at the periphery of the group.

Bacteria have not so far been observed within host cells in these early infection stages, except in a few cases where the leaves have been infected for some time, as is shown in figure 8 where the leaf was inoculated six days previously. In this case there are a few bacteria in a cell beneath the pore cavity. The wall of the cell is hard to define and it is probable that the bacteria gained access to the cell through the ruptured wall or were carried into it by the knife when the sections were cut. Walls of the cells may be invaginated and the nuclei have for the most part become so flattened against them that they are difficult to distinguish from the chloroplasts in fixed and stained preparations (fig. 7e).

Figures 8, 9, and 10 show serial sections of the same leaf in which the bacteria extend from the intercellular spaces beneath the pore cavity into a tracheid. The bacteria are not crowded in the intercellular spaces, and their long axes are pointing in various directions. In the region immediately above the tracheid the bacteria are definitely oriented so that their long axes are directed down the tip of the tracheid.

The next serial section of this same leaf shows a few bacteria in the broader section of the tracheid (fig. 9). In the third section (fig. 10), the bacteria are in the region near the apex of the tracheid. The organisms are so distributed that their individual forms are readily apparent. Nearest the apex they are oriented with their broad surface parallel to the sides of the tracheid, but farther down they are less definitely oriented and their long axes face in various directions.

DISCUSSION

It was early affirmed by Harding and Smith that infection of cabbages by the black rot organism takes place quite naturally by the bacteria entering the specialized water pores at the teeth of the leaves during the damp summer weather. The early investigators all emphasized the importance of moisture in infection and spread of this disease. Garman, who first observed infected plants, noted that periods of high temperature and excessive moisture are favorable for the organism. Pammell also found that plants infected with the organism in rainy weather recovered in dry weather, principally by means of the formation of a corky layer around the

diseased areas. Smith stated that the bacteria grow in the fluid extruded from the water pores during periods of high humidity and move in through the latter in the direction of the food supply.

The experiments which I have carried on confirm the view that a moist atmosphere is necessary if the bacteria are to enter the leaf and bring about infection. Infection took place only when plants had been placed under bell jars and drops of guttation water had collected at the teeth of the leaves before the plants were sprayed. The air in the roof greenhouse where the plants were grown is rather dry and for this reason natural infection of plants was difficult. Cauliflower plants, badly infected in the seedling stages, recovered and produced flowers without any indications of black rot.

The entrance of bacteria into the leaf is dependent upon a continuous pathway of liquid from the tips of the tracheids to the water pores when the plants are very moist and when transpiration is low. It is highly probable under such conditions that the bacteria which may be present at the teeth of the leaf may get into the water pores by their own motility.

If the drop of water at the pore remains for a long time diffusion may possibly aid to some extent in the entrance of bacteria and inert material, but since diffusion rates are generally so slow it is doubtless of little significance alone. Convection currents due to a difference between the temperature within the leaf and the outside air may also be a factor; but in view of the fact that the bacteria have been found considerably advanced in the tissues of the leaf in three hours, it is doubtful that diffusion and convection currents play a prominent rôle in the entrance of the organism.

Entrance of bacteria into the leaf is dependent on a combination of mechanical and biological factors. When evaporation through the stomata is checked due to excessive moisture of the air, surplus water that would ordinarily pass through the stomata in vapor form fills the spaces between cells of the epithem region and appears as droplets at the teeth of the leaves. Bacteria come into contact with this fluid. As the moisture content of the air gradually decreases, transpiration through the stomata is more actively resumed. The drop of water begins to evaporate, its borders begin to recede somewhat in the same fashion as a lake of water drying up, and as the recession continues some of the bacteria may be carried with it towards the pore and finally inside, while others are left stranded behind.

Riker and Ivanoff in experiments involving the entrance of crown gall bacteria into tissues from a wounded surface state that it is possible that capillarity, convection currents, and negative pressure are important factors. They state that due to wounding of the cells in the stem the intercellular spaces are filled with liquid material from the injured cells and the

disease develops in the region that is water-soaked. They conducted experiments with zoogloeae in minute capillary tubes of various shapes to determine the effect of capillary attraction on the movement of this matrix and bacteria, and then attempted to apply the result in explanation of migration of the organisms in tomato stems. It is very difficult, however, to conceive from purely physical grounds how capillary attraction can operate when the intercellular spaces, as they report, are filled with liquid. Were they empty, or filled only with water vapor, the forces of adhesion and cohesion could readily operate and thus attract or draw fluids containing bacteria forward in the host tissues. At least capillary attraction does not seem to be of much significance in the entrance of the black rot organism in the water pores. As noted before, this occurs only when the intercellular spaces are completely filled and the excess water exudes in drops through the hydathodes.

Hill suggested that the fluid released in the tomato stem following the rupture of the cells would be reabsorbed into the surrounding tissue. Ivanoff and Riker claim that this would not be possible owing to the fact that the fluid contains the entire cell contents, not merely the cell sap. They suggest negative pressure as a cause of the rapid movement of the bacteria in the form of zoogloeal strands in entering the tissue. Where the plants are infected by thrusting a needle through the stem on which the bacteria are placed entrance by this means is probable, due to the fact that the air in the stem is rarified. It could not, however, account for the method in which *B. campestre* enters the cabbage leaf through the hydathodes as there is present a continuous pathway of liquid from tracheid tips to pores before the leaves are sprayed with the bacteria, and also because the progress of the bacteria is slower than if negative pressure were operative. Three hours after inoculation the bacteria have been observed among the cells directly beneath the cavity of the hydathodes. They are not in a dense zoogloeal form as reported for *B. amylovorus* and *B. tumefaciens*, but are embedded in a material that stains light blue and which takes the shape of the intercellular spaces.

From the study of freehand sections of the living leaf it is evident that the bacteria retain their motility as they pass from the hydathodes to the tracheid tips. This motility appears to be a more or less darting movement in various directions. As near as can be determined from living, unstained sections, the movement is not definitely oriented in the direction of the tracheids. I have never observed flagella on bacteria in stained slides of infected cabbage leaves.

Multiplication of the bacterial mass or colony appears to be in the direction of the food supply. As the food is exhausted near the hydathode,

multiplication of the bacteria takes place in the direction of the tracheid tips. The organisms are seen densely aggregated in the epithem region where there is liquid between cells. They do not leave this region for the dryer, mesophytic part of the leaf.

In passing through the epithem region to the tracheid tips the bacteria appear to secrete enzymes which dissolve the middle lamellae, and they are thus able to pass between adjacent cells. As a rule, however, the cells in the epithem region are loosely placed in reference to each other. As a consequence the bacteria can doubtless travel for considerable distances without coming into direct contact with cells.

Riker and Ivanoff inoculated plants with India ink and dead bacteria and found that these substances appeared embedded in zoogloeae and moved at approximately the same rate and the same distance as the living crown gall organisms. In an attempt to determine whether or not liquids could enter the cabbage plants through the hydathodes and thus throw some light perhaps on the entrance of bacteria, guttating leaves of my material were soaked in amaranth dye. It was found that the dye entered only at the specialized pores at the teeth of the leaves from which a continuous pathway of liquid extended to the tracheid tips. The dye filled the entire tooth. It did not enter the stomata of the leaves where there was air between the cells. When leaves were soaked in India ink the cabbage juice precipitated the ink particles. There was no evidence that the ink particles together with the bacteria formed zoogloeae as described by Ivanoff and Riker in their work with the tomato. The cells in the epithem of the cabbage leaf are closer together with fewer intercellular spaces than in the tomato tissue studied, however.

Lack of moisture in the stomata, the substomatal cavities, and the intercellular spaces doubtless hinder and prevent the bacteria from entering the stomata on the leaf surface. Smith believed that infection could not take place through the stomata because of the waxy bloom on the leaves of cabbage plants. In various attempts that I have made to infect plants with the organism either by spraying with a suspension of the bacteria on washed leaves or by soaking leaves in a suspension of the organisms, no bacterial invasion was found to take place through the stomata. It seems probable to me that the tissue, although it offers many intercellular spaces, is generally too dry for optimum bacterial development.

This requirement of *B. campestre* for moist tissue is undoubtedly the reason for the scarcity of the disease in dry seasons as noted by earlier investigators. Riker, likewise, found that tumors of the tomato caused by *B. tumefaciens* develop only in the limits of tissue that has been water soaked by wounding or bruising.

One marked difference between my preparations and those of Smith is that I have never found the cavity beneath the pores filled with bacteria. They are always sparsely grouped with their long axes in various directions in spaces between cells just beneath the cavities and in the spaces further down toward the tracheids. The time required for the bacteria to pass from a pore to a tracheid is six or seven days, according to my obervations.

The blue-staining material in which the bacteria lie, I believe to be the fluid of the intercellular spaces of the host plus enzymes and waste products perhaps that are given off by the bacteria. It is not characteristic of the bacteria in the cabbage tissue alone. It is evident in smears of organisms in cabbage broth, in distilled water, and on the surface of leaves which have been sprayed. It is therefore not entirely similar to the material that Bachman reports as being drawn from the cell sap in the pear tissue by *B. amylovorus*.

Doubtless enzymes and bacterial products tend to cause the liquid in which the bacteria lie to stain a blue color. The stain is denser about the organisms that are farthest advanced in the tooth, probably because these organisms are younger and more active than those nearer the water pore.

Nixon, Haber, Riker, Hill and others in studying the migration of *B. amylovorus* and *B. tumefaciens* state that the organisms move through the host tissues in the form of a zoogloea. That is, the bacteria secrete a jelly-like matrix about themselves, and the migrating mass of bacteria or the zoogloea pushes its way through the tissues with its blunt, rounded tip.

The question as to whether or not a zoogloea is present in *B. campestre* is relative, and depends to a certain extent, it seems to me, on one's definition of such a structure. It is impossible thus to draw a sharp line between those with and those without zoogloeae. A zoogloea is generally defined as a gelatinous matrix secreted by the bacteria in which they lie. The consistency of the secreted substance is such as to make flagellar motion difficult or impossible. In the wide range of bacterial forms almost any degree of variation in the consistency of the gelatinous matrix may be found. The most extreme case is perhaps in the Myxobacteriaceae where the secretion is so copious that they are able to build definite symmetrical bodies. In forms such as *B. amylovorus* it is less abundant, and so on down the line to species like *B. campestre* where the secretion is comparatively slight. This latter organism does not lose its motility in the time it takes for the organism to reach the tracheids, as may be ascertained by examining hand sections of infected leaves. The bacteria move about in the spaces between cells so that their long axes point in various directions. They are not oriented in one direction as reported by Hill for *B. tumefaciens*. On

this account it may be deduced that the fluid in which *B. campestre* lies is not exceedingly thick or jelly-like. It is none the less slightly denser than the liquid in the intercellular spaces, in the triple stain its limits are occasionally visible. Ordinarily, the liquid in which the bacteria lie completely fills the intercellular spaces. At least it does not precede the bacteria to any appreciable extent as is clearly evident where lamellae have been partially dissolved.

Smith reported that *B. campestre* forms a zoogloea in fluid exuded from water pores. I interpret this substance to be the same as that in which the bacteria lie within the cabbage plant. It is not dense enough to show convex tips and does not push its way between cells, as far as my observations go.

The bacteria have a very definite effect on host cells. In plants fixed three hours after spraying the protoplasts of cells with bacteria about them appear somewhat collapsed, and the outlines of the nuclei are difficult to follow. In cells 7 days after inoculation little cytoplasm can be seen. The nucleoli have disappeared. The nuclei have either collapsed against the walls or have disappeared. That liquids have been lost from the cells is evident from their lack of turgidity and invaginated walls. Plasmolysis is never complete, however, as the cytoplasm maintains its position around the cellulose walls. In certain cells a few bacteria are seen. I believe that they have been carried in by the knife when the sections were cut or that the walls have disintegrated after an infection of 6 days allowing them to enter. This effect of *B. campestre* on the host tissue is thus very similar to that reported for *B. amylovorus*, *B. tabacum* and *B. vignae* by the investigators noted above. *B. campestre* does not cause the abnormal formation of cells as described for *B. radicicola* and *B. tumefaciens*.

Entrance to the tracheids appears to be at their spiral tips. Often the bacteria may be seen lying along the lateral wall, but they do not gain entrance to the vessel. A number of investigators have assumed that the bacteria dissolve their way through the tracheid walls by means of enzymes. Here, the bacteria without any great evidence of mass action appear to pass through the tips of the tracheids.

The action of *B. campestre* in the above study appears thus to be quite different from the infection of horse-radish by what is said to be a new variety of *Bacterium campestre* reported by McCulloch in 1929. The organism causes leaf spots of horse-radish, and less readily of cabbage and related plants. Infection is usually stomatal. The node of infection and the action of the bacteria in the tissues as reported by McCulloch are very different from the action of the typical black rot organism in the cabbage plant.

CONCLUSIONS

1. Infection of plants by *Bacterium campestre* takes place when a continuous pathway of liquid from the tips of the tracheids to the hydathodes is present.

2. Entrance of bacteria into the leaf is dependent on a combination of biological and mechanical factors. Motility of the organisms, diffusion, and convection currents may influence to a slight degree the entrance of the bacteria. Recession of drops of water contaminated with the bacteria from the hydathode when transpiration is resumed in the plant is doubtless an important factor.

3. In their passage from the hydathodes to the tracheid tips the bacteria travel only in the epithem region where a fluid pathway is present. They retain their motility and growth is in the direction of the food supply, which is the tracheid tips. Here also, however, passage of the organisms is facilitated by recession of the liquid material of the epithem region as water evaporates from the leaf. Their ability to secrete enzymes that dissolve the middle lamellae makes passage between cells possible.

4. The organism secretes products that give a blue color to the fluid of the intercellular spaces when stained with the triple stain. The fluid in which the bacteria lie is but slightly denser than the fluid of similar regions in uninfected plants.

5. The organism causes invagination of cell walls, disappearance of nucleoli, the collapse of nuclei and chloroplastids against cell walls, and a decrease in the amount of cytoplasm of the cells.

I am very grateful to Professor R. A. Harper, with whom this study was begun, for his stimulating interest and advice, to Professor J. S. Karling for his assistance and criticism, and to Professor S. F. Trelease for his helpful suggestions.

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Explanation of plates 9-12

(All figures drawn with aid of camera lucida)

Plate 9

Fig. 1. (×800) Cross section of tooth of cabbage leaf showing
- a. water pore cavity
- b. specialized water pore or hydathode
- c. terminus of tracheid.

Plate 10

Fig. 2. (×1200) Bacteria lying in a film of blue-staining material above a stoma.

Fig. 3. (×1600) Cross section of hydathode region of cabbage leaf 3 hours after inoculation
- a. The bacteria are wedged between cells.

Fig. 4. (×1200) Bacteria among cells beneath pore cavity. 7½ days after inoculation.

Fig. 5. (×1200) Cells of epithem region 6 days after inoculation. Bacteria have destroyed the middle lamellae and are present in the narrow passages between cells. The chloroplasts and nuclei have collapsed against the walls and in certain cells they have disappeared.

Plate 11

Fig. 6. (×1200) Bacteria are in the spaces between the cells
- a. wall of cell invaginated by bacteria
- b. terminus of tracheid.

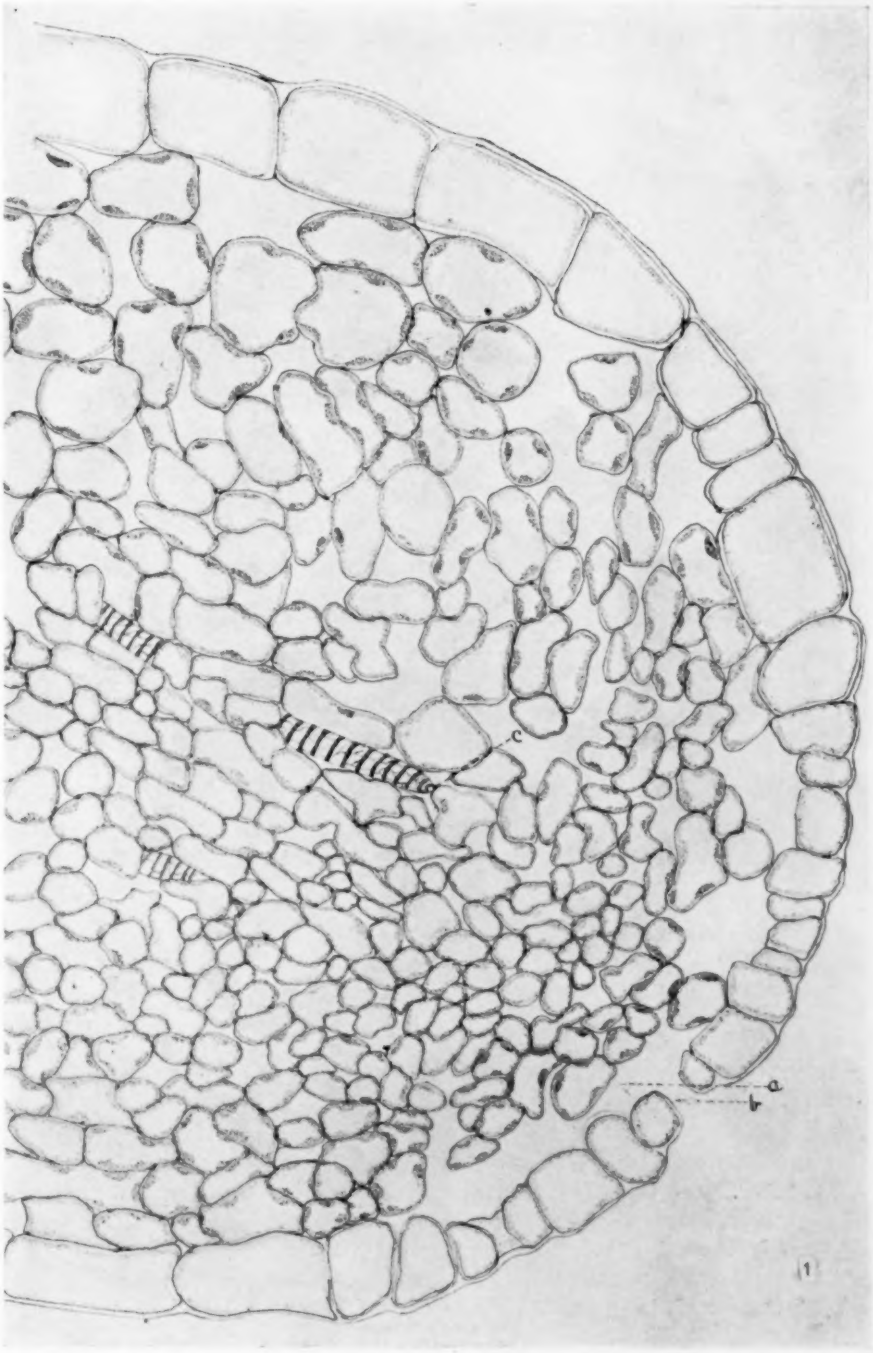
Fig. 7. (×1200)
- a. Cell of uninfected plant from region bordering a pore
- b. Cell of uninfected plant from epithem region
- c. Cell from region bordering the pore of plant infected 6 days
- d, e. Cells from epithem region of plant infected 6 days
- f. Cell from region bordering pore of plant infected 3 hours.

Plate 12

Fig. 8. (×1200) Water pore region of tooth. Bacteria lie in the intercellular spaces of cells and are entering the tip of a tracheid. 6 days after inoculation.

Fig. 9. (×1200) Serial section to Fig. 8. Bacteria in the tracheid.

Fig. 10. (×1200) Serial section to fig. 9. Bacteria are in the tracheid.



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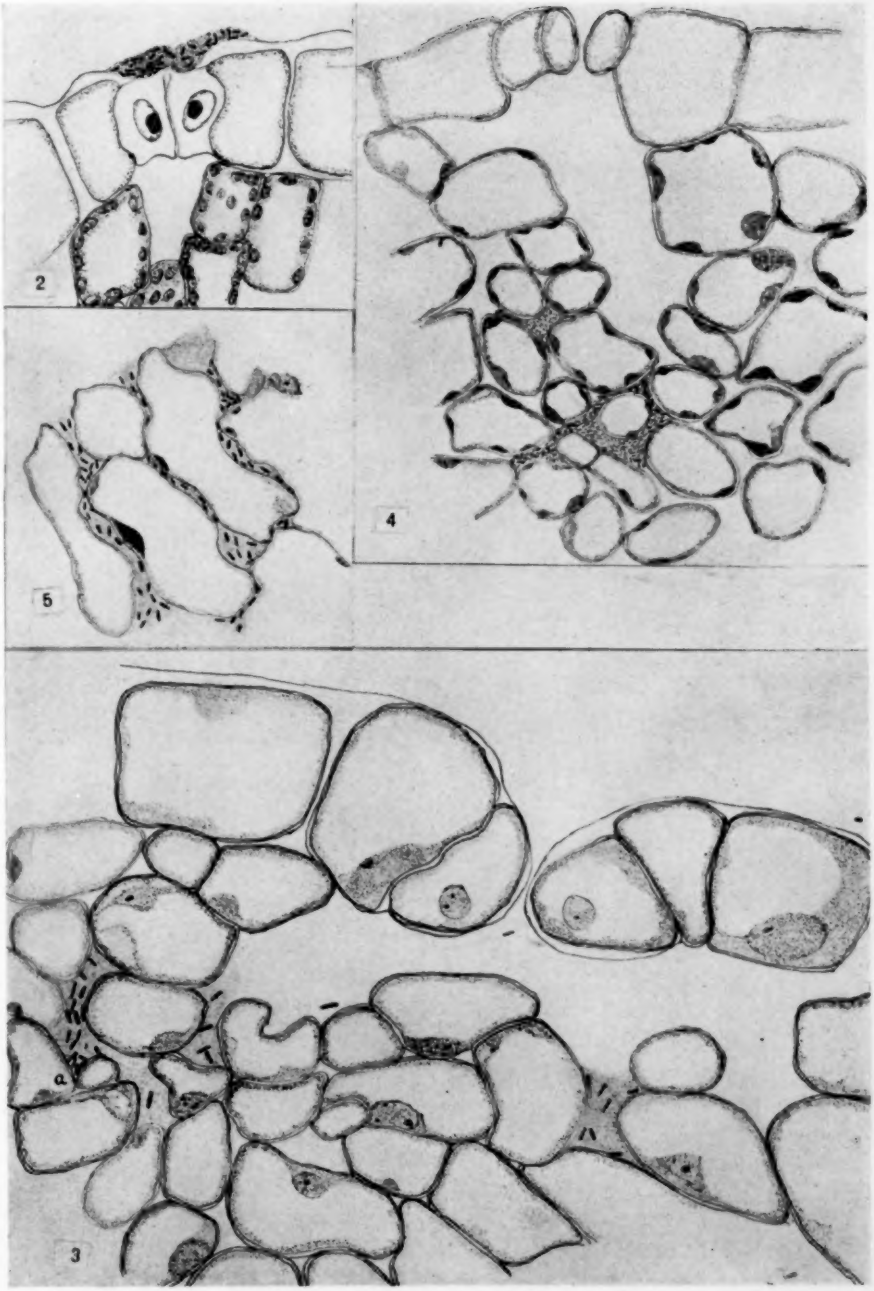

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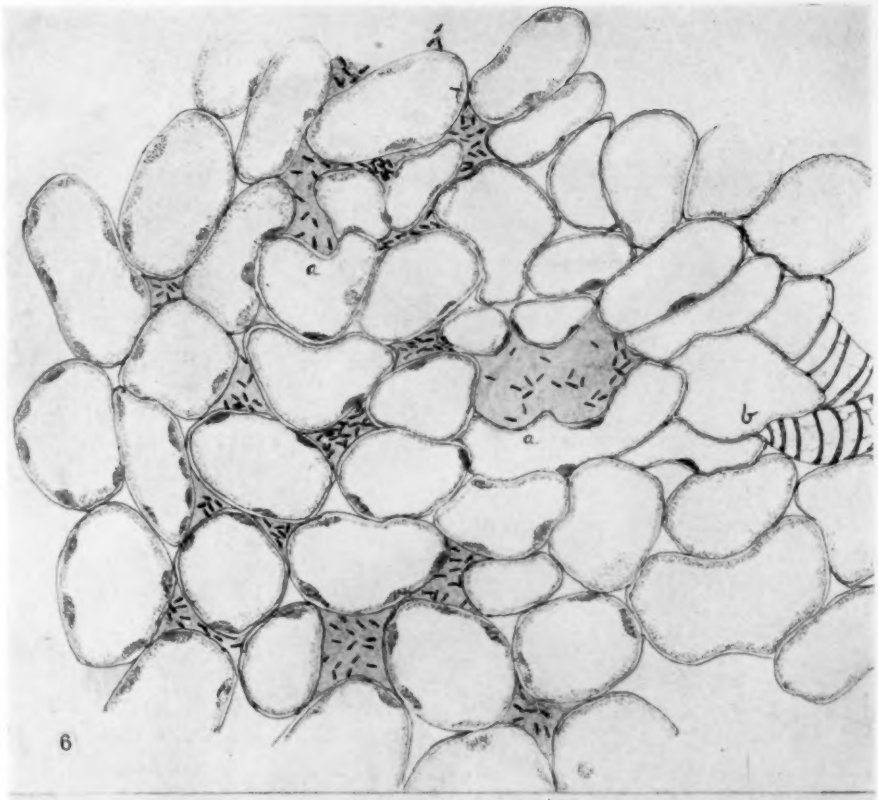

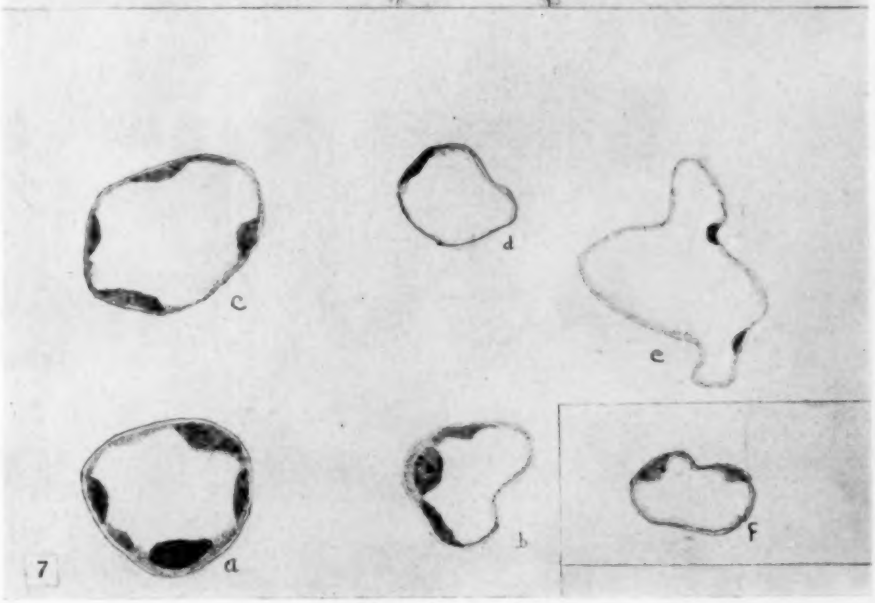

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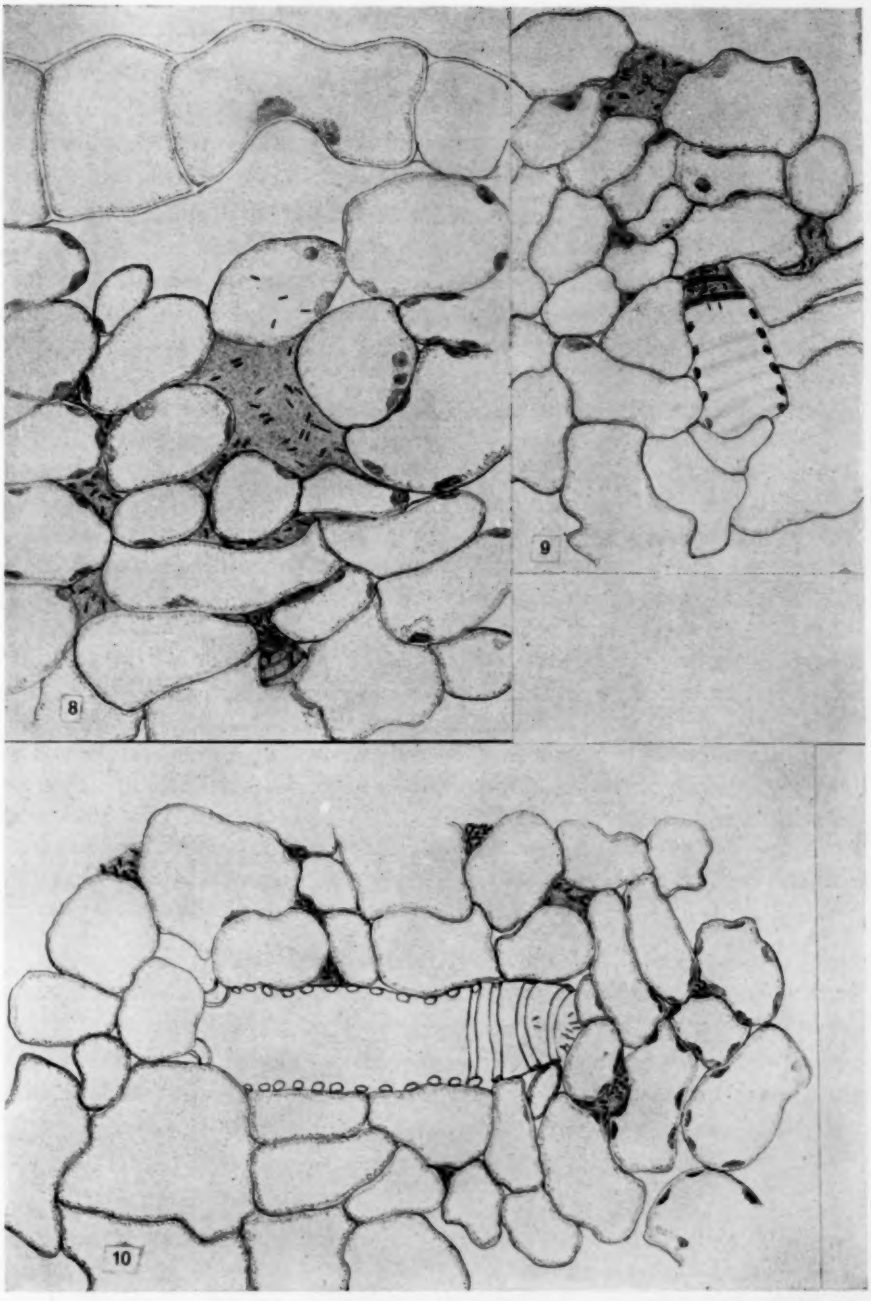

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Plantae Krukovianae II

H. A. Gleason and A. C. Smith

During 1932, several hundred flowering plants, mostly woody, were collected in the vicinity of the Maracassumé River, State of Maranhaõ, Brazil, by Mr. R. Froes, under the direction of Mr. B. A. Krukoff. The several new species disclosed by this collection, together with one previously incompletely known species, are described in the present paper. All types are deposited in the herbarium of the New York Botanical Garden.

LAURACEAE

Aniba opaca A. C. Smith, sp. nov. Arbor ad 10 metralis; ramis ramulisque teretibus fuscis, juventute cinereo-puberulis mox glabris; petiolis crassis rugosis supra canaliculatis 6–11 mm. longis; laminis coriaceis glabris siccitate fuscis opacis oblongis, 12–18 cm. longis, 3–6 cm. latis, basi cuneatis, apice breviter acuminatis, margine integris et leviter recurvatis, costa utrinque prominente, nervis secundariis utroque 8–12 adscendentibus supra planis subtus prominulis, venulis reticulatis subplanis; inflorescentiis ad apices ramulorum congestis, ad 10 cm. longis et 30-floris, ramulis subteretibus et floribus cinereo-tomentellis; floribus pedicellatis (pedicellis 1–2 mm. longis) bracteolis parvis mox deciduis subtentis, maturitate circiter 3 mm. longis et diametro; perianthii tubo obconico lobos subaequante, lobis 6 aequalibus late ovatis obtusis pellucido-punctatis intus glabris, circiter 1.5 mm. longis et latis; staminibus 9 fertilibus, staminodiis nullis; serierum exteriorum filamentis glabris quam antheris paullo brevioribus, antheris late ovoideis obtusis, locellis minutis apertis; seriei tertiae filamentis basi glandulis binis sessilibus auctis, antheris subglobosis; ovario glabro ellipsoideo sub anthesi 1 mm. longo, stylo rugoso ovarium aequante, stigmate subtruncato.

Type, *Froes 1730*, collected May 6, 1932, at Ubim, Maracassumé River region, State of Maranhaõ. A local name is "Louro Abacate" and the tree is used for lumber. It is related to *A. guyanensis* Aubl., than which it has shorter stouter petioles, fewer secondary veins, and larger flowers. It also bears a close superficial resemblance to *A. rosaeodora* var. *amazonica* Ducke, but has its leaves strictly glabrous rather than tomentellous beneath, and its ovary glabrous and comparatively long-styled.

Ocotea Froesii A. C. Smith, sp. nov. Arbor ad 12 metralis, trunco ad 20 cm. diametro; ramis ramulisque crassis conspicue angulatis arcte flavo-cinereo-velutinis demum teretibus et glabris; petiolis crassis rugosis velutinis supra canaliculatis 8–17 mm. longis; laminis coriaceis siccitate fuscis ovato-oblongis, 15–30 cm. longis, 6–13 cm. latis, basi cuneatis, apice acuminatis, margine integris et leviter recurvatis, supra glabris subtus densissime et minutissime sericeo-tomentellis, costa subtus prominente, nervis secundariis utroque 5–7

arcuato-adscendentibus supra prominulis subtus elevatis, venulis reticulatis subtus prominulis; inflorescentiis solitariis axillaribus multifloris quam foliis brevioribus, ramulis angulatis densissime cinereo-tomentellis; floribus (in ramulis brevibus dense aggregatis) hermaphroditis extra arcte cinereo-tomentellis pedicellatis (pedicellis circiter 1.4 mm. longis) sub anthesi 2–2.5 mm. longis et 4 mm. diametro; perianthii tubo subnullo, lobis 6 subaequalibus oblongis intus glabris, 1.5–2 mm. longis, 1 mm. latis, apice obtusis; staminibus 9 fertilibus, antheris oblongis obtusis 0.4 mm. longis; serierum exteriorum filamentis glabris quam antheris paullo brevioribus; seriei tertiae filamentis prope basin glandulis binis sessilibus auctis; ovario glabro ellipsoideo sub anthesi 1 mm. longo, stylo carnoso ovarium fere aequante; bacca subglobosa ad 1 cm. diametro rugosa, cupula obconica carnosa margine integra.

Type, *Froes 1790*, collected July 16, 1932, on "terra firma" in the Maracassumé River region, State of Maranhaõ. A local name is "Louro do Igapó." It is a species of the Section *Mespilodaphne*, in which the angled branchlets, large leaves, close sericeous pubescence, and spreading oblong perianth segments distinguish it. It is allied to *O. Beyrichii* (Nees) Mez, which differs, in addition to the above characters, by having staminodes.

ROSACEAE

Moquilea riparia Gleason, sp. nov. Arbor 12 m. alta, ramis juvenilibus castaneis glabris lenticellis parvis notatis; petioli crassi rugosi minutissime puberuli 5–8 mm. longi; foliorum laminae subcoriaceae oblongae vel obovato-oblongae usque ad 11 cm. longae 6 cm. latae ad apicem apiculatam rotundatae ad basin rotundatae vel truncate vel paullulum cordulatae utrinque glabrae supra subnitentes subtus opacae, costa supra leviter elevata subtus prominente, venis secundariis in utroque latere 10–15 leviter arcuato-adscendentibus supra obscuris subtus paulum elevatis, venulis utrinque obscuris; stipulae jam delapsae; paniculae laterales vel subterminales 10 cm. longae, axibus cano-pubescentibus, ramis lateralibus 4–6, 2–6 cm. longis; flores numerosi in racemos spiciformes laterales et terminales dispositi, fasciculis plerumque 3-floris, bracteolis lanceolatis pubescentibus pedicellos 0.8–1 mm. longos vix aequantibus; hypanthium poculiforme vel late turbinatum 1.6 mm. altum 3 mm. in diametro extra breviter cano-pubescens intus araneosum; sepala reflexa triangularia 1.1.2 mm. longa lataque cano-pubescentia; petala patentia suborbicularia 2 mm. longa fere glabra; stamina circ. 20 horizontaliter patentia, filamentis gracilibus glabris 2–3 mm. longis, antheris late ellipsoideis dorsifixis 0.5 mm. longis; ovarium centrale ovoideum 1 mm. longum dense araneosum, ovulis 2 a basi adscendentibus; stylus basalis gracilis 4 mm. longus fere usque ad apicem araneoso-villosus.

Type, *Froes 1961*, collected 20 Oct. 1932, in sandy soil in *terra firma*, Maracassumé River region, Maranhaõ. *Froes 1935*, from *varzea* land in

the same region is the same; its local name is "Camacary." Apparently only six petaliferous species of *Moquilea* have been described hitherto. Of these, five are sharply distinguished from ours by obvious characters. Specimens of the sixth, *M. elata* Pilger, have not been examined; according to the description it differs from ours in its leaves opaque on both sides and in its elliptic ciliate petals. It was collected on the upper Rio Acre, more than two thousand miles from Maranhaõ, and in all probability is not conspecific with *M. riparia*.

MELIACEAE

Trichilia Froesii A. C. Smith, sp. nov. Arbor ad 20 metralis, trunco prope basin 20 cm. diametro; ramis ramulisque cinereis striatis mox glabris; foliis 15–25 cm. longis, petiolis supra canaliculatis basi incrassatis 1.5–3 cm. longis, juventute arcte cinereo-puberulis mox glabris, rhachidibus velut petiolis vel subteretibus, foliolis plerumque 9 alternis, petiolulis incrassatis rugosis decidue puberulis 1–2 mm. longis (foliolis terminalibus ad 12 mm. longis), laminis subcoriaceis oblongis, 7–15 cm. longis, 2–5 cm. latis, basi cuneatis, apice obtuse acuminatis ad apiculum minutum callosum, margine integris vel subcrenulatis, utrinque fuscis praeter costam interdum puberulam glabris, supra nigropunctatis, costa supra leviter elevata subtus prominente, nervis secundariis utroque 12–14 rectis patulis prope margines adscendentibus supra planis subtus elevatis vel planis, venulis copiose reticulatis subplanis; paniculis multifloris axillaribus solitariis pedunculatis saepe folia subaequantibus, ramulis striatis cinereo-puberulis; floribus breviter pedicellatis, extra subtilissime cinereo-puberulis; calyce cupuliformi, dentibus 5 deltoideis obtusis; petalis 5 valvatis oblongis carnosis, circiter 3 mm. longis et 1.5 mm. latis, apice acutis incurvatis; filamentis connatis, tubo subcarnoso glabro 1.5 mm. longo, margine inter antheras dentes 0.5 mm. longos gerente; antheris 10 ovoideis 0.8 mm. longis; ovario sessili late conico, dense et arcte luteo-hirsuto, 3-loculari, loculis 2-ovulatis, ovulis collateralibus; stylo carnoso breviter cylindrico, stigmate truncato.

Type, *Froes 1917*, collected Sept. 16, 1932, at Mata da Cachoeira, Maracassumé River region, State of Maranhaõ. It is a species of the Section *Moschoxylum*, related to *T. japurensis* C. DC., from which it differs by having the leaflets black-punctate and with fewer secondary nerves, and the filament tube glabrous rather than puberulent within.

SAPINDACEAE

Cupania olivacea Gleason & Smith, sp. nov. Sect. *Trigonocarpus* Radlk. Frutex vel arbor parva; ramuli sulcati mox teretes, primum ferrugineo-tomentelli demum glabri; folia 15–20 cm. longa, petiolo subtereti 4–9 cm. longo basi incrassato primum tomentello mox glabro, rhachide petiolo simili, foliolis alternis 6–8 subcoriaceis supra sicco olivaceis subtus fuscis, petiolulis subcarno-

sis rugosis supra leviter canaliculatis 2–4 mm. longis, laminis oblongis vel obovato-oblongis, 8–13 cm. longis, 3.5–4.5 cm. latis basi acutis apice obtusis vel leviter emarginatis margine integris vel levissime crenulatis utrinque glabris, costa supra plana vel impressa subtus valde prominente, nervis secundariis utroque 7–10 patentibus supra leviter elevatis subtus prominentibus, venulis utrinque elevatis et conspicue reticulatis; paniculae in axillis superioribus solitariae 7–20 cm. longae ad anthesin tomentellae; flores 2–3 in glomerulos subsessiles conferti bracteis 2–3 chartaceis oblongis 3 mm. longis et 1 mm. latis extra dense pilosis intus glabris subtenti; pedicelli 1 mm. longi dense pilosi; sepala 5 subcarnosa oblonga 3.5 mm. longa et 2 mm. lata extra dense pilosa intus glabra; petala 5 praeter squamas glabra membranacea orbicularia, ungui gracili 1 mm. longo, lamina 2–2.5 mm. diametro rotundata basi 2-squamata, squamis adscendentibus subdeltoideis circa 1 mm. longis utrinque pilosis; discus carnosus annularis; stamina 8 intra discum, filamentis erectis carnosis circa 3 mm. longis infra medium pilis pallidis 0.8 mm. longis ornatis supra medium angustatis, antheris oblongis 1 mm. longis glabris; ovarium in floribus ♂ sterile subconicum 1–2 mm. longum dense strigulosum; sepala in fructu persistentia; capsula late pyriformis trialata purpureo-brunnea 15 mm. longa et 18 mm. diametro apice late truncata basi in stipitem brevem angustata, extra leviter pilosula praesertim ad basin, septis angustis glandulis minutissimis arcte notatis; semina atra ellipsoidea 7 mm. longa 4 mm. diametro.

Type, *Froes 1978*, collected Oct. 22, 1932, at Campo de Boa Esperança, Maracassumé River region, State of Maranhaõ. A local name is "Páo de Arapuce." It is most closely related to *C. racemosa* (Vell.) Radlk., in comparison with which it has subentire rather than serrate leaflets, larger flowers with a longer and paler pubescence, and petals which are more definitely orbicular, more slenderly clawed, and with rounded rather than subacute scales. Young ♀ flowers of the new species have not been observed.

ELAEOCARPACEAE

Sloanea reticulata A. C. Smith, sp. nov. Arbor ad 15 metralis, trunco 15 cm. diametro; ramis ramulisque teretibus cinereis glabris, juventute arcte puberulis et lenticellatis; foliis plerumque prope apices ramulorum congestis, petiolis gracilibus teretibus decidue tomentellis 1–3 cm. longis basi et apice incrassatis; laminis papyraceis glabris (juventute nervis parce tomentellis) ovatis, 10–18 cm. longis, 5–9 cm. latis, basi obtusis vel rotundatis saepe subcordatis, apice obtusis vel breviter acuminatis (apice ipso saepe emarginato vel calloso-mucronulato), margine undulatis, nervis secundariis utroque 6–9 patulis cum costa supra planis subtus prominentibus, venulis copiosissime reticulatis utrinque prominulis; inflorescentiis solitariis axillaribus quam foliis multo brevioribus (ad 6 cm. longis) cymulosis 3 (vel 2)-floris (floribus rare solitariis epedunculatis), pedunculis teretibus rectis 2–4 cm. longis tomentellis,

pedicellis velut pedunculis 10–20 mm. longis prope basin bracteolas lanceolatas 1.5 mm. longas mox deciduas gerentibus; sepalis 4 ovatis acutis, 7–8 mm. longis, 4–5 mm. latis, utrinque densissime arcte puberulis; toro 5 mm. diametro; staminibus numerosissimis quam calyce brevioribus, filamentis gracilibus 1 mm. longis dense setosis, antheris (appendiculis filiformibus ad 1.5 mm. longis inclusis) circiter 3.5 mm. longis parce puberulis; ovario ovoideo sub anthesi 3 mm. longo pilis ad 0.8 mm. longis densissime setoso, loculo unico, placentis 4 parietalibus ad margines interiores ovulos multos gerentibus, stylo gracili ovarium aequante glabro.

Type, *Froes 1918*, collected Sept. 16, 1932, at Mata da Cachoeira, Maracassumé River region, State of Maranhaõ. A local name is "Guabiraba branca." It is related to *S. Garckeana* Schum., from which it is distinguished by the fine reticulate venation of the larger thinner leaves and by the short inflorescences. *S. Garckeana* has a much thinner flagellate anther tip and placentas completely fused to form a 4-celled ovary, while the placentas of the new species are quite separate at their inner edges.

LECYTHIDACEAE

Couratari coriacea Mart. Two collections of Froes are referred to this species, previously described from fruit only.[1] The pyxidium of Martius' original collection seems to agree perfectly with those of the present collection; it is exceptionally thin in texture, slender, and with an uncontracted mouth. The species is also noteworthy because of its obtuse leaves and the small number of stamens, which are arranged in a single row. The following description is based on the cited specimens:

Arbor ad 45 metralis, trunco ad 2 m. diametro; ramis ramulisque fuscis rugosis glabris lenticellatis; petiolis juventute supra puberulis et canaliculatis 10–15 mm. longis; laminis coriaceis siccitate fuscis oblongis, 6–9 cm. longis, 3.5–4.5 cm. latis, basi apiceque obtusis vel rotundatis, margine integris vel obsolete crenulatis, utrinque glabris, costa prominente, nervis secundariis utroque 10–13 patulis prope margines anastomosantibus supra planis subtus elevatis, venulis inconspicue reticulatis; inflorescentiis post defoliationem maturantibus racemosis ut videtur ramulos breves terminantibus, 3–9 cm. longis, rhachidibus arctissime puberulis, pedicellis gracilibus maturitate ad 12 mm. longis cum calycibus velut rhachidibus puberulis; calycis tubo obconico, sepalis 6 aequalibus carnosis ovatis, circiter 2 mm. longis et 3 mm. latis, basi connatis, apice rotundatis, margine fimbriatis; petalis aequalibus membranaceis juventute puberulis obovatis, circiter 2 cm. longis et 1 cm. latis; androphoro explanato 25 mm. longo, ligula 5–9 mm. lata, galea carnosa 8–10 mm. diametro appendiculis numerosissimis anantheris filiformibus imbricatis obtecta, staminibus circa annulum 10–14, antheris subsessilibus ovoideis 0.5 mm. longis;

[1] Berg; Mart. Fl. Bras. 14[1]: 510, *pl. 75, f. 2.* 1858, Miers, Trans. Linn. Soc. 30: 283. 1875.

ovario omnino infero, vertice plano, stylo brevi obtuso, loculis 3; pyxidio cylindrico-trigono-conico (pedicello gracili 3 cm. longo), 8–9 cm. longo, pericarpio coriaceo subtenui (1–2 mm. crasso) extra rugoso longitudine 12–15-costato intus septorum 3 vestigiis pallidis signato, zona calycari 3–3.5 cm. diametro, vitta interzonali erecta 6–10 mm. longa, operculo subrugoso 3–3.5 cm. diametro plano leviter umbonato, columella crassa triquetra; seminibus circiter 12 a pyxidio compressis oblongis, 6.5–7 cm. longis, 1.5–1.8 cm. latis, scuto embryonifero centrali, ala membranacea cincto.

Maranhaõ: Maracassumé River region, Candido Mendes, *Froes 1763* (leaves and flowers); Mata da Cachoeira, *Froes 1901* (flowers and fruits). It is locally known as "Tauary branco," and the bark is said to furnish a fiber used for caulking boats.

SAPOTACEAE

Pouteria ovata A. C. Smith, sp. nov. Arbor, ramulis cinereis teretibus glabris rugosis; foliis glabris, petiolis gracilibus rugosis 12–20 mm. longis supra canaliculatis, laminis coriaceis nitidis ovato-oblongis, 9–14 cm. longis, 5–6.5 cm. latis, basi cuneatis, apice breviter acuminatis, margine integris anguste revolutis, costa utrinque prominente, nervis secundariis utroque 10–12 patulis prope margines adscendentibus utrinque elevatis, venulis reticulatis subtus prominulis; inflorescentiis axillaribus, pedunculo communi crasso 1–5 cm. longo densissime ferrugineo-sericeo, floribus tetrameris 6–10 in glomerulos aggregatis, pedicellis 2–5 mm. longis velut pedunculo pubescentibus; sepalis utrinque arctissime puberulis subaequalibus late ovatis, circiter 1.5 mm. longis et 2 mm. latis, exterioribus carnosis fuscis, interioribus pallidis; corolla membranacea extra parce puberula intus glabra 2–2.5 mm. longa, lobis tubum aequantibus orbicularibus circiter 1 mm. latis margine imbricatis; staminibus in medio corollae affixis, antheris subsessilibus ovoideis subacutis 0.7 mm. longis per rimas laterales dehiscentibus; staminodiis carnosis deltoideis acutis quam antheris paullo brevioribus; ovario depresso-globoso sub anthesi 1–1.5 mm. diametro pallide striguloso, loculis 2, ovulo in quoque loculo unico, stylo carnoso glabro 1 mm. longo; fructis cinereis glabris ellipsoideis, circiter 2 cm. longis et 1.5 cm. latis (pedicellis fructu ad 7 mm. longis), pericarpio 2 mm. crasso, semina unica.

Type, *Froes 1841*, collected Aug. 31, 1932, on high rocky land near sea coast, Ilha do Trauira, Maracassumé River region, State of Maranhaõ. It is a species of the Section *Pseudocladia*, related to *P. lateriflora* (Benth.) Radlk. and *P. cladantha* Sandwith. From the former it differs in the shape of its leaves, which are decidedly broader in proportion and cuneate rather than acute at base, and in its shorter pedicels which give the inflorescence a more compact appearance. From *P. cladantha*, the new species differs by its long petioles and its more prominent secondary nerves, and by its glabrous differently shaped fruit.

THE NEW YORK BOTANICAL GARDEN

Cytology of a translocation of the 1·2 chromosome in Datura

A. Dorothy Bergner and A. F. Blakeslee

(with figures 1–16)

Simple translocations form one of the kinds of chromosomal change brought about by radiation treatment. A chromosome is broken into two parts, one of which is translocated to a host chromosome while the other part remains as a free fragment. Among the 80 prime types,[1] which have been rendered homozygous for modified chromosomes, about 20 per cent have been classified as due to simple translocations. The first of this kind of chromosomal change found in *Datura* involved the 1·2 which is the largest (L) chromosome. A brief note concerning the translocation was published earlier.[2] It was mentioned in a dicussion of prime types[1] where it was listed as PT6. The present paper will give an account of the cytology of this translocation while another paper will discuss the unbalance brought about by the products of the translocation when present as extra material.

During the summer of 1928 a cytological examination was made of some plants which had been obtained following radiation treatment. Included in the group was the plant number 27377(1), which was normal in appearance. The 24 chromosomes, instead of showing the arrangement of twelve bivalents at metaphase I in pollen-mother-cells, were grouped into eight bivalents plus a circle of four and a chain of four chromosomes. It is with the chain of four chomosomes only that we are concerned in the present paper. There was only one unmodified 1·2 chromosome; the equivalent of the other 1·2 chromosome was found in a free fragment and a fragment which was translocated to another chromosome.

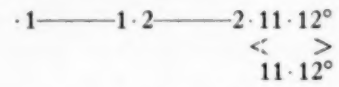
Presumably as a result of radiation treatment, the 1·2 chromosome was broken into two parts, the break occurring in the region where the spindle fiber is inserted.[3] Crosses with appropriate testers have shown that it was the ·1 half which remained free as a rod-shaped chromosome. At

[1] Bergner, A. D., S. Satina and A. F. Blakeslee, 1933. Prime types in Datura. Proc. Nat. Acad. Sci. **19**: 103–115.

[2] Bergner, A. D., S. Satina, A. G. Avery and A. F. Blakeslee. 1929. Translocated Sugarloaf, a pure-breeding chromosomal type in *Datura* induced by radium treatment. Science **70**: 562.

[3] Confusion has arisen because of the numerous senses in which the word "attachment" is used. Following Sharp and some of his associates, we are using the expression "insertion of the spindle fiber" instead of "attachment of the spindle fiber." This is equivalent to Belling's[4] phrase "fusal attachment." The word attachment has also been used in regard to unions between non-homologous chromosomes (translocations) by such workers as Painter and Muller,[5] Dobzhansky[6] and Belling[4]; to unions

one end it has the spindle fibre which formerly was inserted submedianly in the 1·2 chromosome that was broken. The ·2 half was translocated to the 11·12° chromosome in the order 2·11·12°. The chain of four chromosomes found in 27377(1) is explainable on the basis that like ends are attached and may be represented as follows:



In this and other diagrams in the text, the broken line represents points where expected attachments often are lacking. The 11·12° chromsome is of M size; when the ·2 fragment is united with it the size of the 2·11·12° chromosome is slightly larger than the 1·2 or L chromosome. The ·12 end of the 11·12° chromosome is characterized by a terminal hump designated by the sign ° previously employed in other publications. In the actual chromosomes shown in the figures, adjacent humps join to form a single compound hump. When there is no attachment between the two ·11 ends, a chain of four chromosomes is formed as shown in figure 1*a*. When these ·11 ends are attached, however, a closed bivalent is formed by the two 11·12° chromosomes and a "kite-like" configuration of four chromosomes results as shown in figure 1*b*. Except figure 14, all metaphase I chromosomes have been drawn from acetocarmine preparations of pollen-mother-cells. Except fig. 14, all have been drawn at a magnification of about 1700 diameters. The numbers of the ends of those chromosomes which are being discussed in the text have been added to the drawings, so that the latter will be more intelligible to the reader.

In appearance, the offspring of this plant resembled either diploids or the 2n+2·2 secondary type called "Sugarloaf." Among the latter group, some were recorded as weakly Sugarloaf while others were indistinguish-

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between homologous chromosomes (attached X's in *Drosophila*); and to temporary attachments between similar ends of chromosomes at diakinesis and metaphase. For the latter type of attachment Belling[7] has used the term "terminal junction." Ultimately it may be desirable to adopt a more precise terminology for the different senses now covered by the word attachment.

[4] Belling, J. 1933. Crossing over and gene rearrangement in flowering plants. Genetics **18**: 388–413.

[5] Painter, T. S. and H. J. Muller. 1929. The parallel cytology and genetics of induced translocations and deletions in *Drosophila*. Journ. Hered. **20**: 287–298.

[6] Dobzhansky, Th. 1932. Studies on chromosome conjugation. I. Translocations involving the second and the Y-chromosomes of *Drosophila melanogaster*. Zeit. für Ind. Abst. und Vererb. **60**: 235–286.

[7] Belling, J. 1933. Critical notes on C. D. Darlington's "Recent advances in cytology." Univ. Cal. Publ. Bot. **17**: 75–110.

able from the 2n+2·2 type. Cytologically six types could be distinguished among these offspring.

There were three types among the diploids. First there were those which showed a "kite" or chain of four chromosomes like the parent. They were heterozygous PT6, which may be represented as follows:

$$\cdot 1 \text{———} 1 \cdot 2 \text{———} \begin{matrix} 2 \cdot 11 \cdot 12^\circ \\ < \quad > \\ 11 \cdot 12^\circ \end{matrix}$$

Another type had 12 bivalents like those of our standard Line 1 shown in figure 2. They may be represented as follows:

$$\begin{matrix} 1 \cdot 2 \\ < \quad > \\ 1 \cdot 2 \end{matrix} + \begin{matrix} 11 \cdot 12^\circ \\ < \quad > \\ 11 \cdot 12^\circ \end{matrix}$$

The third type also showed 12 bivalents but was distinguishable from the Line 1 diploids because of the appearance of two of the bivalents. Being homozygous PT6, this type may be represented as follows:

$$\cdot 1 \text{———} 1 \cdot + \begin{matrix} 2 \cdot 11 \cdot 12^\circ \\ < \quad > \\ 2 \cdot 11 \cdot 12^\circ \end{matrix}$$

In figure 3, the $(\cdot 1)_2$ fragments can be recognized as a pair of rod-shaped chromosomes attached to one another at one end only, in contrast to the usual pairs of V-shaped chromosomes which are attached at both ends. The $2 \cdot 11 \cdot 12^\circ$ pair is also distinguishable because the $(\cdot 2)_2$ fragments show as a projection from the closed bivalent formed by the $(11 \cdot 12^\circ)_2$ host chromosomes as shown in figure 3*a*. If the $\cdot 11$ ends fail to attach, the $(2 \cdot 11 \cdot 12^\circ)_2$ pair forms a large circle as shown in figure 3*b*.

The Sugarloaf types contain excess $\cdot 2$ chromosomal material. Cytologically two types were distinguished which were weakly Sugarloaf in appearance. The one type showed two bivalents joined by the translocated fragment as shown in figure 4. It may be represented as follows:

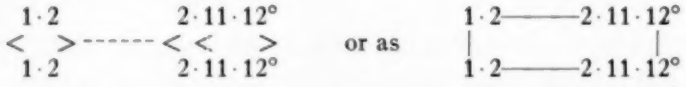
$$\begin{matrix} 1 \cdot 2 \\ < \quad > \\ 1 \cdot 2 \end{matrix} \text{———} \begin{matrix} 2 \cdot 11 \cdot 12^\circ \\ < \quad > \\ 11 \cdot 12^\circ \end{matrix}$$

The other type showed a "kite-like" configuration as shown in figure 5 and represented as follows:

$$\cdot 1 \text{———} 1 \cdot 2 \text{———} \begin{matrix} 2 \cdot 11 \cdot 12^\circ \\ < \quad > \\ 2 \cdot 11 \cdot 12^\circ \end{matrix}$$

Both types have the $\cdot 2$ half of the $1 \cdot 2$ chromosome in excess over the normal chromosomal material.

The third type of Sugarloaf is morphologically indistinguishable from the 2n+2·2 secondary type because it has two ·2 halves of the 1·2 chromosome in excess. Cytologically, however, the two are easily distinguished. The secondary 2n+2·2 has 25 chromosomes whereas this Sugarloaf type obtained from 27377(1) has 24 chromosomes. In a previous publication[1] it was listed as PT5. It is shown in figure 6 and may be represented as follows:



Prime type 5 may be further distinguished from the secondary type through breeding behavior. Having 24 chromosomes, it is pure-breeding, whereas the secondary 2n+2·2 Sugarloaf, like all 25 chromosome types, throws both normals and its own type among its offspring.[8]

[8] Blakeslee, A. F., A. D. Bergner and A. G. Avery. 1933. Methods of synthesizing pure-breeding types with predicted characters in the jimson weed, *Datura stramonium*. Proc. Nat. Acad. Sci. **19**: 115–122.

Explanation of figures 1–6

Fig. 1 *a* and *b*. Chromosomal configuration of plants which are heterozygous for the translocation: (*a*) chain of four chromosomes formed when there is no attachment between the two ·11 ends; (*b*) "kite-like" configuration of four chromosomes formed when the ·11 ends are attached. The numerical formula for these four chromosomes is ·1—1·2—2·11·12°—°12·11. In (*b*) the ten bivalents have been omitted.

Fig. 2. Twelve bivalents found in normal diploids (standard Line 1).

Fig. 3 *a* and *b*. The two bivalents of homozygous PT6 which are different from bivalents found in normal diploids (standard Line 1). In (*b*) there is no attachment between the two ·11 ends. The bivalents are $(\cdot 1)_2$ and $(2\cdot 11\cdot 12°)_2$.

Fig. 4 *a* and *b*. Chromosomal configuration of a weakly Sugarloaf type which contains the ·2 half of the 1·2 chromosome as excess material. In (*a*) the ·2 ends of the two 1·2 chromosomes are not attached; in (*b*) they are. In (*b*) the ten bivalents have been omitted. The formula for this type, which is heterozygous PT5, is $(1\cdot 2)_2$——2·11·12°—°12·11.

Fig. 5. Chromosomal configuration of the second weakly Sugarloaf type. Its formula is ·1—1·2—$(2\cdot 11\cdot 12°)_2$. The ten bivalents have been omitted.

Fig. 6 *a*, *b*, *c*, and *d*. Chromosomal configurations of plants homozygous for PT5, a 24-chromosome, pure-breeding Sugarloaf type. In (*a*) the $(1\cdot 2)_2$ bivalent is connected with the $(2\cdot 11\cdot 12°)_2$ bivalent; the ·11 ends are attached. In (*b*) and (*d*) the bivalents are separate. In (*b*) the ·11 ends are attached. In (*c*) the four chromosomes have opened out to form a large circle. The ten bivalents have been omitted in (*a*) (*b*) and (*c*).

Figs. 1–6

In the previous paragraphs are given the chromosomal constitutions of the original translocation and the different derivatives from it. How the ends have been identified may be seen from the following paragraphs.

The primary $2n+11\cdot12^{\circ}$ plant heterozygous for PT5 (which it will be shown has the chromosome $2\cdot11\cdot12^{\circ}$) showed a chain of five chromosomes as illustrated in figure 7*a*. Therefore the M-sized $11\cdot12^{\circ}$ chromosome is involved in this translocation. In figure 7*a*, an M-sized bivalent and an L-sized bivalent are joined together by a chromosome slightly larger than L and somewhat bipartite. The M-sized bivalent is obviously $(11\cdot12^{\circ})_2$; the L-sized bivalent is $(1\cdot2)_2$. The connecting chromosome must consist of an $11\cdot12^{\circ}$ chromosome to which the fragment was translocated, the point of union being indicated by a slight constriction, hence the bipartite appearance. The $\cdot12$ end of the $11\cdot12^{\circ}$ chromosome has a terminal hump as represented by a ° sign. Since the fragment was translocated to the end which is opposite the humped or $\cdot12^{\circ}$ end, it must have been translocated to the $\cdot11$ end. This is clearly indicated in figure 7*b*. This connecting chromosome, therefore, must be $X\cdot11\cdot12^{\circ}$, the X representing the translocated fragment. The chain of five shown in figure 7*a* may be represented as follows:

$$\begin{matrix}2\cdot1\\ <\ >\\ 2\cdot1\end{matrix}\text{———}\quad\text{or}\quad\begin{matrix}1\cdot2\\ <\ >\\ 1\cdot2\end{matrix}\text{———}X\cdot11\cdot12^{\circ}\text{———}\begin{matrix}^{\circ}12\cdot11\\ <\ >\\ ^{\circ}12\cdot11\end{matrix}$$

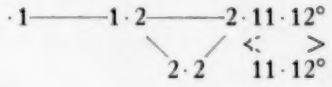
This conclusion that the $11\cdot12^{\circ}$ chromosome is the host chromosome and that the fragment was translocated to the $\cdot11$ end was verified by crossing PT5 to PT11. The latter has the modified chromosomes $11\cdot13$, $^{\circ}12\cdot17$, $14\cdot18$ instead of the Line 1 chromosomes $11\cdot12^{\circ}$, $13\cdot14$ and $17\cdot18$.[1] The $14\cdot18$ chromosome is recognizable because of its miniature size, the $^{\circ}12\cdot17$ because of its being 1-sized with a terminal hump at the $\cdot12^{\circ}$ end. The configuration of eight chromosomes induced by this cross consists of a circle of six connected with an L or $1\cdot2$ bivalent and may be represented as follows:

$$\begin{matrix}18\cdot17\text{———}17\cdot12^{\circ}\text{———}^{\circ}12\cdot11\cdot X\\ |\qquad\qquad\qquad\qquad\quad|\\ 18\cdot14\text{———}14\cdot13\text{———}13\cdot11\end{matrix}\text{———}\begin{matrix}2\cdot1\\ <\ >\\ 2\cdot1\end{matrix}\quad\text{or}\quad\text{———}\begin{matrix}1\cdot2\\ <\ >\\ 1\cdot2\end{matrix}$$

This circle sometimes breaks into a chain as shown in figure 8*b*. That chromosome in the circle which connects $^{\circ}12\cdot17$ with the $(1\cdot2)_2$ bivalent must therefore be $^{\circ}12\cdot11\cdot X$ as shown in the above diagram. The $\cdot11$ end of the $11\cdot13$ chromosome connects with the original $\cdot11$ end of the $^{\circ}12\cdot11\cdot X$ chromosome, leaving the translocated fragment as a projection from this circle as shown in figure 8*a*. It is this X fragment which connects the $(1\cdot2)_2$ bivalent with the circle.

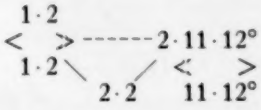
Proof will now be given that the fragments come from the 1·2 chromosome and that it is the ·2 half which was translocated to the 11·12° chromosome. It was indicated by the size of the fragments and by their attachments to the L chromosomes, when they were heterozygous, that they are parts of the 1·2 chromosome. This conclusion was confirmed by the evidence which determined the identity of the fragments.

The secondary 2n+2·2 plant heterozygous for PT6 shows a chain of five chromosomes. The 2·2 chromosome was identified by a tendency to bend back on itself to form a "doughnut," a characteristic of secondary chromosomes. This method of using a secondary chromosome to identify the ends of a modified chromosome has been described in a previous publication.[1] In figure 9, the 2·2 chromosome may be seen lying between the 1·2 or L chromosome and the X fragment which was translocated to the 11·12° chromosome. Diagramatically it may be represented as follows:

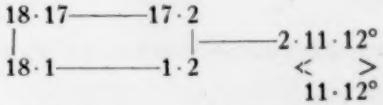


Therefore this X fragment has the ·2 end of the 1·2 chromosome and the modified chromosome is correctly written as 2·11·12°.

Prime type 5 may be used in the test as well as PT6. A secondary 2n+2·2 plant heterozygous for PT5 may show the 2·2 chromosome lying between a 1·2 and the X·11·12° chromosome in a chain of five. However, since there are two 1.2 chromosomes they tend to form an independent pair and the chain therefore is usually broken. This is the case in figure 10 which illustrates this cross. The same argument used for identifying the ends in a 2n+2·2 plant heterozygous for PT6 applies to a 2n+2·2 plant heterozygous for PT5, the diagram for which may be written as follows:



A cross between PT5 and PT2, the so-called "B" race which has the chromosomes 1·18(m) and 2·17(L) instead of the Line 1 chromosomes 1·2(L) and 17·18(m), results in a configuration of six chromosomes consisting of a circle of four to which an M-sized bivalent is attached. This is shown in figure 11. It can be proved to have the attachment shown in the following diagram:



The fragment translocated to the 11·12° chromosome is attached to the circle of four between the two L chromosomes 1·2 and 2·17, thus showing that it possesses a ·2 end.

Several independent lines of argument, therefore, have shown that it is the ·2 fragment which was translocated to the 11·12° chromosome. From inspection of figure 1 (which is an example of heterozygous PT6), it will be seen that the free fragment must be ·1 since it is attached to the

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Explanation of figures 7–15

Fig. 7 *a* and *b*. Chromosomal configurations found in a 2n+11·12° plant heterozygous for PT5. In (*a*) the 2·11·12° chromosome connects the two closed bivalents $(1\cdot 2)_2$ and $(11\cdot 12°)_2$; the ten bivalents have been omitted. In (*b*) a chain of three chromosomes $(11\cdot 12°)_2$—°12·11·2 is shown, the eleven bivalents having been omitted.

Fig. 8 *a* and *b*. Configurations of eight chromosomes found in a plant heterozygous for PT5 and PT11. In (*a*) the $(1\cdot 2)_2$ bivalent is open; in (*b*) the circle is broken at the ·11 attachment points. The formula of this configuration is $(1\cdot 2)_2$—2·11·12°—°12·17—17·18—18·14—14·13—13·11.

Fig. 9. A chain of five chromosomes found in a 2n+2·2 plant which is heterozygous for PT6. The 2·2 chromosome forms a "doughnut" between the 1·2 and 2·11·12° chromosomes. The chain is represented as ·1—1·2—2·2—2·11·12°—°12·11.

Fig. 10 *a* and *b*. Chromosomal configurations found in 2n+2·2 plants which are heterozygous for PT5. In (*a*) the 2·2 chromosome is attached to the ·2 end of the 2·11·12° chromosome; in (*b*) it is attached to the $(1\cdot 2)_2$ bivalent. These broken chains have the formula $(1.2)_2$—2·2—2·11·12°—°12·11. The ten bivalents have been omitted.

Fig. 11. Configuration of six chromosomes found in a plant heterozygous for PT2 and PT5. Formula for this configuration is 11·12°—°12·11·2—2·17—17·18—18·1—1·2.

Fig. 12. Chain of five chromosomes found in a 2n+1·2 plant heterozygous for PT6. The free fragment is attached to the chain at the ·1 ends of the $(1\cdot 2)_2$ chromosomes. The formula is ·1—$(1\cdot 2)_2$—2·11·12°—°12·11.

Fig. 13. Chromosomal configuration of a 2n+·1 plant. The ·1 fragment is attached to the $(1\cdot 2)_2$ bivalent.

Fig. 14. The $(1\cdot 2)_2$ bivalent drawn at a magnification of 2900 diameters to show that one arm (·1?) is slightly longer than the other (·2?).

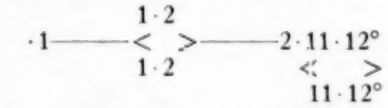
Fig. 15. Chromosomal configuration of a pure-breeding 26-chromosome plant $\left(\frac{\cdot 1}{2\cdot 2}\right)_2$. The $(\cdot 1)_2$ bivalent is recognized as a pair of rod-shaped chromosomes; the two attached 2·2 chromosomes form a "dumbbell."

Fig. 16. Group of four chromosomes found in the 24-chromosome $\left(\frac{1\cdot 1}{2\cdot 11\cdot 12°}\right)$ compensating type. The formula is 1·1—1·2—2·11·12°—°12·11.

Figs. 7–16

1·2 chromosome at the ·1 end. Additional evidence regarding the identity of the free fragment follows.

The primary 2n+1·2 type heterozygous for PT6 shows a chain of five chromosomes in figure 12. The free fragment is attached to the chain between the two 1·2 chromosomes at a point known to be the ·1 end because of the orientation of that 1·2 chromosome which lies next to the 2·11·12° chromosome. The free fragment is easily recognized by the attenuated appearance of its unattached end. The 11·12° chromosome is recognized by its size and by the hump at the ·12° end; the 2·11·12° chromosome by its size, appearance and position in the chain. The L or 1·2 chromosomes must have the orientation given in the following diagram:



The fragment which is attached to the $(1 \cdot 2)_2$ chromosomes must have a ·1 end on account of its position in the configuration.

Morphological evidence that this free fragment is the ·1 half of the 1·2 chromosome was demonstrated in a plant which had this fragment as excess material. In appearance the plant resembled somewhat the 2n+1·1 secondary type but it departed less widely from normal. Its chromosomes are shown in figure 13.

Datura chromosomes have median or sub-median spindle fiber insertion regions, with the result that they are V-shaped at metaphase I. Terminal attachment points are at each end of these chromosomes. When the 1·2 chromosome was broken, the ·2 fragment became united by its inner surface at the point of the break to the 11·12° chromosome. The attachment point at the ·2 end therefore is free. Moreover the union of the ·2 fragment to the ·11 end was slightly subterminal so that the ·11 attachment point also is free. Therefore the 2·11·12° chromosome has three attachment points instead of the usual two. The functioning of all three of these points is shown in figures 3*a* and 6*a–b* when two 2·11·12° chromosomes are present and in figures 1*b*, 4*a–b*, 8*a*, 10*a* and 16 when only one is present. Attachment often is lacking between the two ·11 ends; this leaves only the ·2 and ·12° ends functioning as shown in figures 3*b*, 5 and 6*c–d* when two 2·11·12° chromosomes are present and in figures 7*a*, 8*b*, 9, 11 and 12 when only one is present.

The $(\cdot 1)_2$ chromosomes form a rod-shaped pair united by the attachment points at the ·1 end of each. The opposite end of each is distinguishable by being somewhat ragged and drawn out as though the end of the coiled chromonema were loosened and pulled away from the body of the

chromosome by the spindle fiber which is inserted here. This end was formerly the mid-region of the 1·2 chromosome which was broken.

Familiarity with the 1·2 or L chromosome has shown that the point at which the spindle fiber is inserted is slightly sub-median. The two arms of this V-shaped chromosome therefore are somewhat unequal. In figure 14 this 1·2 chromosome is shown drawn from sectioned material that had been fixed in a modified Carnoy and stained with Heidenhain's hematoxylin. Cytological evidence favors the ·1 arm as the longer in the 1·2 chromosome.

Continued use of the 1·1 and 2·2 secondaries as testers in crosses with prime types indicates that the 1·1 chromosome is the longer. Thus the 1·1 chromosome in figure 16 appears to be longer than the 2·2 chromosomes in figures 9, 10 and 15. Definite cytological proof that this is the case requires the presence of both the 1·1 and 2·2 chromosomes in the same pollen-mother-cell. By proper breeding procedure, we are attempting to obtain such a plant with the two secondaries together. If secondary chromosomes are formed by the doubling of a single arm in all cases, the apparent size differences found in these secondaries is indirect evidence that the ·1 arm in the 1·2 chromosome is the longer.

In all the five figures in which both the free and the translocated fragments are shown together in the same pollen-mother-cell, the ·1 fragment appears to be the longer. The size difference may be slightly exaggerated on account of the fact that the ·1 fragment has an attachment at one end only.

In *Datura*, as in other forms, the accumulating evidence seems to indicate that at least the majority of chromosomal deficiencies are lethal when homozygous. Prime type 6, which is homozygous for both the free and the translocated fragments, is viable. From the assumed effects of deficiencies, it would follow, therefore, that no part of the 1·2 chromosome was lost as a consequence of the translocation under consideration. Apparently the break occurred at the point where the spindle fiber is inserted, the spindle fiber going with the free fragment.

The ·1 fragment can compensate with the 2·2 chromosome to furnish the equivalent of the 1·2 chromosome which is lacking entirely in the pure-breeding type $\left(\frac{\cdot 1}{2\cdot 2}\right)_2$ shown in figure 15. The ·2 fragment can compensate with the 1·1 chromosome to furnish the equivalent of a 1·2 chromosome in the compensating type $\frac{1\cdot 1}{2\cdot 11\cdot 12^{\circ}}$ as shown in figure 16. The diagram of the latter may be represented as follows:

$$1\cdot1\text{———}1\cdot2\text{———}2\cdot11\cdot12^{\circ}$$
$$\lessdot \quad \gtrdot$$
$$11\cdot12^{\circ}$$

From these compensations it is evident that the $\cdot1$ fragment supplies the chromosomal material which is lacking in the $2\cdot2$ chromosome to furnish a complete $1\cdot2$ chromosome; and that the translocated $\cdot2$ fragment similarly supplies what is lacking in the $1\cdot1$ chromosome. Together the $1\cdot1$ and the $2\cdot2$ chromosomes form the equivalent of a $1\cdot2$ chromosome plus additional material. If, when taken together, these two secondary chromosomes left a deficiency for the $1\cdot2$ chromosome, only one and not both of the two compensating types given above would be expected. In consequence a $1\cdot1$ plus a $2\cdot2$ chromosome might be equivalent to more than two $1\cdot2$ chromosomes but could not be equal to less.

The exact mechanism involved in the formation of a secondary chromosome is not understood and need not concern us in the present discussion. It is sufficient to say that the process in some way assures a locus for the insertion of a spindle fiber in the middle of the double-ended chromosome that results.

In view of the evidence available, we believe that the breakage resulting in the translocation under discussion was at essentially the same point where the breakage which resulted in the $1\cdot1$ and the $2\cdot2$ secondary chromosomes took place; and that this point corresponds to the locus of the spindle fiber insertion which separates the $1\cdot2$ chromosome into two unequal arms.

SUMMARY

As a result of radiation treatment there was produced a gamete in which the $1\cdot2$ chromosome was broken in the mid-region. The $\cdot2$ half was translocated to the $\cdot11$ end of the $11\cdot12^{\circ}$ chromosome, forming the $2\cdot11\cdot12^{\circ}$ chromosome. The $\cdot1$ half remained free as a rod-shaped chromosome.

Cytologically three types of normal-appearing plants were found in the offspring: those like normal diploids from which the translocation arose, those which were heterozygous and those which were homozygous for the translocation (PT6). The latter has the formula: $(\cdot1)_2+(2\cdot11\cdot12^{\circ})_2$.

Cytologically two types which had a single extra dose of $\cdot2$ material were distinguished: $(1\cdot2)_2+2\cdot11\cdot12^{\circ}+{}^{\circ}12\cdot11$ and $\cdot1+1\cdot2+(2\cdot11\cdot12^{\circ})_2$. They resembled the $2n+2\cdot2$ secondary type called "Sugarloaf."

A true-breeding Sugarloaf type (PT5) was obtained: $(1\cdot2)_2+(2\cdot11\cdot12^{\circ})_2$. It cannot be separated from the $2n+2\cdot2$ secondary by external appearance but it is easily distinguished both cytologically and genetically.

The translocation of the ·2 half to the 11·12° chromosome was sufficiently subterminal to permit the functioning of the terminal attachment point at the ·11 end. The 2·11·12° chromosome therefore has three attachment points.

The rod-shaped ·1 fragment shows attachment only at that end which is the ·1 end of the former 1·2 chromosome. The other end of this fragment has the spindle fiber of the broken 1·2 chromosome, the break having occurred apparently at the point where the spindle fiber is inserted in the 1·2 chromosome.

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Flowering and seed production in Amphicarpon floridanum[1]

PAUL WEATHERWAX

(WITH TEXT FIGURES 1–9)

Cleistogamy has arisen in the Gramineae in several different ways, and probably the most highly specialized of these occurs in three species which are known to bear fertile spikelets on underground shoots. Pursh described the first of these as *Milium amphicarpum* in 1814. This was afterwards placed in a new genus and has since been known in the manuals as *Amphicarpon*[2] *Purshii* Kunth or *A. amphicarpon* (Pursh) Nash. A second species, *A. floridanum*, was later described by Chapman. In a recent monograph[3] on *Paspalum* Mrs. Chase describes a West Indian species, *P. amphicarpum* Ekman, which has a similar underground inflorescence.

The taxonomic descriptions state that ordinarily only the underground spikelets of Amphicarpon are fertile. Bentham and Hooker[4] and Baillon[5] even go so far as to consider the plant monoecious, the aerial spikelets being described as staminate and those underground as pistillate. The impossibility of pollination under these conditions apparently did not occur to them. Chapman[6] says that the aerial flowers are rarely fruitful. Mrs. Chase[7] reports that she found a few fruits in aerial inflorescences of *A. amphicarpon* which were observed in the field. In *Psapalum amphicarpum* both the aerial and the subterranean inflorescences produce fruits.

This seems to comprise all that has been published on the flowering and fruiting of these species, and it is obvious that little of this information has come from observations made in the field. I have had opportunity in the past few years to examine *Amphicarpon floridanum* in most stages of its life history, and some interesting facts may now be added to what has been known.

Material.—One interested in this species need never be limited by lack of material, for it is found in great abundance in many parts of Florida. It is common along roads and railroads, in the edges of fields, and in open pasture land in all the central part of the state where the soil is suitable, and thousands of acres of the prairie region north of Lake Okeechobee are covered with it. I have found the best flowering material along road grades across the marshes just west of Groveland and on the calcareous sandy

[1] Publication No. 63 of the Waterman Institute, Indiana University.

[2] Also spelled *Amphicarpum*.

[3] Contrib. U. S. Nat. Herb. **28**: 1–310. 1929.

[4] Genera Plantarum. **3**: 1099. 1883.

[5] Histoire des Plantes. **12**: 304. 1894.

[6] Flora of the Southern U. S. 1884.

[7] Bot. Gaz. **45**: 135–136. 1908.

soil north and east of Lake Okeechobee, and even in the streets of Okeechobee.

Large quantities of material have been examined in the field, and herbarium specimens and fixed material have also been studied in the laboratory. Living plants have been kept for several years in the greenhouse, where they usually flower from October to January. The plant seems intolerant of acidity in the soil and grows best in the presence of a definite alkalinity (pH 8.2 to 8.6).

Description.—The culms arise from an extensive system of branching rhizomes. Most of them are sterile, and there is a great variation from place to place in the abundance of flowering material. I have never found subterranean spikelets on plants that did not have also aerial inflorescences, and in many instances only the latter are present. The underground spikelets are frequently overlooked by the collector unless he is unusually careful or knows the peculiarity of the plant with which he is dealing.

The aerial inflorescence is a simple panicle bearing from three or four to 15 or 20 spikelets (fig. 1). The shoots bearing the underground spikelets arise singly or in clusters from near the base of the aerial culm or from elsewhere on the rhizome. Each of these shoots (fig. 4) bears a number of scale leaves and is terminated by a single spikelet. Other details can be seen in the figures.

The spikelets are much like those of the other Paniceae. The lower floret is represented by only an empty lemma. The first glume is minute in the aerial spikelet and obsolete in the underground spikelet (figs. 2–4). Other differences between the two are correlated with the conditions under which they develop and with their behavior at the time of flowering.

The aerial spikelet and flower have in general the structure common to grasses that are pollenzied by the wind (figs. 2, 3, 5, 6). The lodicules, which are large and well developed, function very effectively, pushing the lemma and palea wide apart, and the dorsal anther emerges on a long filament and opens and releases its pollen. The two lateral stamens, however, usually remain enclosed by the palea whose inrolled edges will not permit them to be exserted (figs. 5, 8). These imprisoned anthers release their pollen before the emergence of the stigmas, so that close pollination might normally occur if the floret did not open at all. Any stigma that fails to be effectively pollenized in this way has, of course, other chances when the dorsal anthers of flowers in other spikelets emerge. In some cases two or all three of the stamens of a flower may emerge as in typical grasses.

The regular abortion of most of the potential fruits of the aerial spikelets is almost certainly due to some factor operating at the time of flowering, because development stops at this point. My plants in the greenhouse

regularly produce a considerable number of well developed fruits, although other grasses usually produce fruits less freely under these conditions than in their normal habitats. This suggests some deficiency in the development of the pollen tube, the more humid atmosphere of the greenhouse probably being sufficient to insure success. It should be noted also that spikelets containing mature fruits readily disarticulate from their pedicels, and, consequently, data obtained from herbarium specimens will ordinarily not indicate a true state of affairs.

The subterranean spikelet is a little larger than the aerial one at the time of flowering, and the parts of the floret are much modified (fig. 7). The ovary is large, but the stigmas are small, distorted, and rudimentary. The anthers are small and short and almost sagittate in form. The anthers are closely applied to the stigmas and the whole mass is tightly wedged into the conical cavity formed at the top between the lemma and palea. The lodicules are small and apparently make no progress at all toward opening the floret. Consequently close pollination necessarily occurs.

The fruit formed underground is much larger than that of the aerial panicle. All attempts to germinate the seeds of either kind have resulted in failure, although dissection leaves no doubt of their viability, and seedlings developing from the underground seeds are frequently found.

Discussion.—This plant gives unusually good evidence as to the trend toward cleistogamy in certain types of grasses. It is not a very long step from the basal cleistogamous shoots of *Danthonia*, *Sporobolus*, etc., to the specialized shoots of this genus. These modified shoots are probably the reduced remnants of axillary branches which were once terminated by panicles. In the ancestors of *Amphicarpon* these branches may have been of the nature of the basal branches of the autumnal phase of many species of *Panicum*.

The aerial floret of *Amphicarpon* also suggests a possible step in the evolution of cleistogamy. The inrolling of the edges of the stiff, indurated palea makes it so difficult for the anthers to emerge that two of them usually remain included. A slight additional induration at the base of the lemma and palea would be sufficient to prevent the hinge-like action necessary for their separation, and this condition seems to have been reached in the cleistogamous spikelets growing underground. The subsequent degeneracy of the lodicules and decrease in the size of the anthers and stigmas would be expected, because, although we have no entirely satisfactory mechanical explanation of it, the correlation between cleistogamy and the degeneracy of lodicules, anthers, and stigmas, is too consistent to be attributed wholly to coincidence.

Figs. 1–9. *Amphicarpon floridanum.*

Fig. 1. Habit.

Figs. 2, 3. Aerial spikelets at anthesis.

Fig. 4. Cleistogamous, underground spikelet.

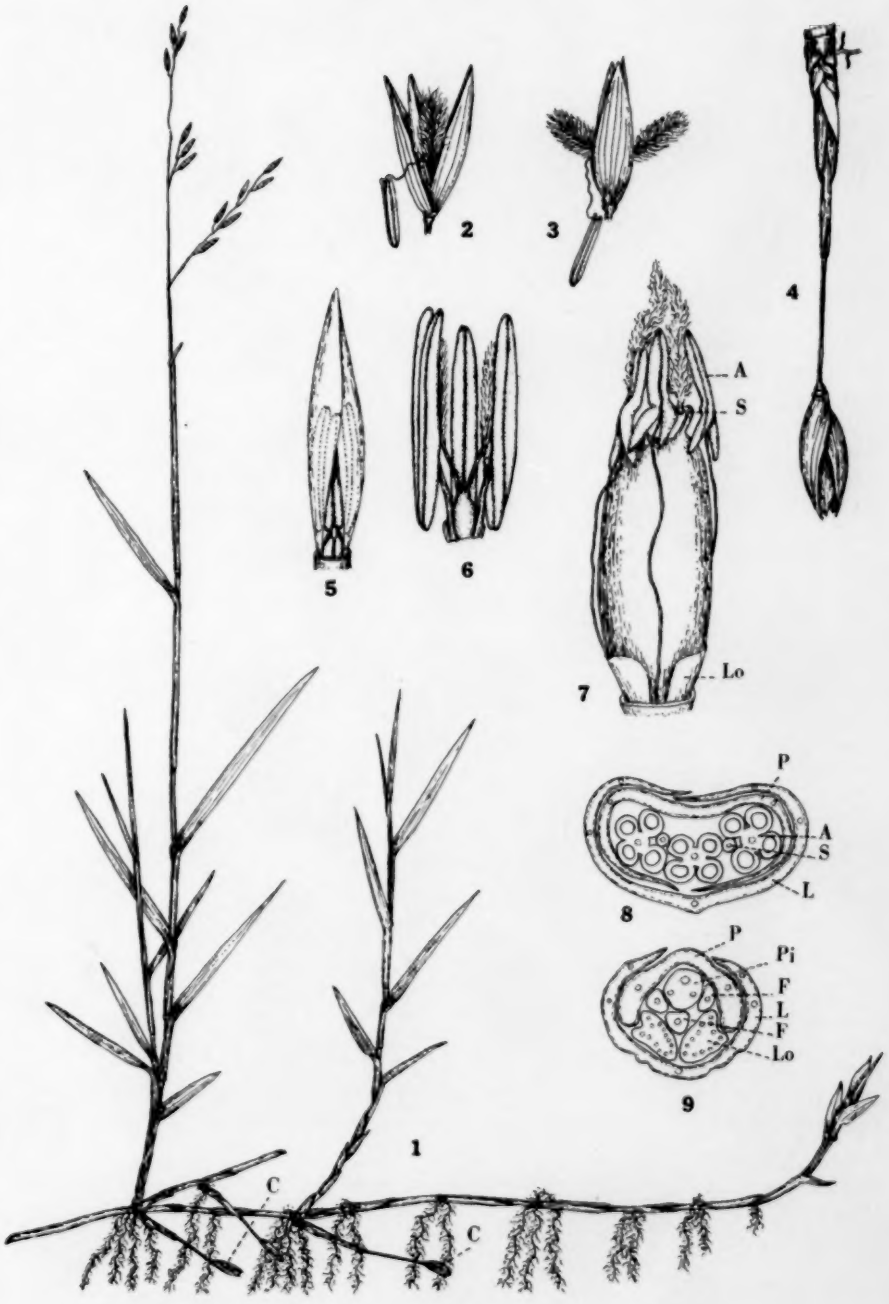
Fig. 5. Dorsal view of aerial floret with lemma removed.

Fig. 6. Ventral view of aerial flower.

Fig. 7. Dorsal view of cleistogamous flower.

Figs. 8, 9. Sections through aerial floret at different levels.

C, cleistogamous spikelet; A, anther; S, style; Lo, lodicule; P, palea; L, lemma; Pi, pistil; F, filament of stamen.



Figs. 1–9

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Possible importance of respiration water to young wheat seedlings[1]

BURTON E. LIVINGSTON

That the respiratory process produces water as well as carbon dioxide has long been known, but the possible importance of water so derived has received but little attention. Although the amount of water metabolically formed in an experiment period is doubtless usually small when compared with the amount of water absorbed from the environment in the same period, nevertheless, respiration water is highly important in physiological theory and it surely plays a really considerable rôle in the water supply of some organisms under some sets of environmental conditions.

This topic interested de Saussure, who dealt with it in 1804. Liaskovskii's studies on seed germination, reported in 1874, are usually mentioned in recent writing on metabolic water. Liaskovskii was acquainted with the earlier studies of Oudemans and Rauwenhoff, but only through Knüttel's apparently rather complete abstract (Knüttel 1859–60). I have not seen the original Dutch paper (Oudemans and Rauwenhoff 1858), but Knüttel's German article reports that these authors made extensive experiments (about 1857) on the chemical changes that accompany seed germination, concluding, among other interesting things, that water was actually produced, as well as carbon dioxide. It appears that Babcock's discussion of this subject published in 1912, is the most thorough and the most recent, although I may have missed some later references. In 1924 Kostychev wrote, in his monograph on plant respiration (p. 11): "Die Wasserbildung bei der Pflanzenatmung ist leider unzureichend untersucht worden. Dies ist um so mehr zu bedauern, als namentlich die Ausgiebigkeit der Wasserbildung in manchen Fällen als Kennzeichen einer totalen Verbrennung des Atmungsmaterials dienen kann." He then referred to Liaskovskii's studies, pointing out that hydrolytic phenomena complicate the interpretation of such experiments, and his short paragraph ends with the words, "trotzdem ist es dringend notwendig, die so wichtige Lücke auszufüllen." Nothing was added in 1926, when the same author's text (p. 460) on chemical plant physiology appeared, nor in Lyon's translation of it (p. 380), published in 1931, excepting that Lyon cited Bonnier's (1893) researches to emphasize the importance of hydrolysis in seed germination. In Raber's recent elementary text book (1933, p. 294) the reports of Liaskovskii and Babcock are mentioned but the author seemed to think that metabolic water in plants is scarcely worth considering at all, because its hydrogen and oxygen must necessarily represent water previously reduced in carbohydrate formation; to regard the production of respiration water as a part

[1] Botanical contribution from The Johns Hopkins University, no. 125.

of the plant's water supply would be "arguing in a circle," as Raber thought. It is not necessary to forget, however, that the vast majority of plant forms (bacteria, fungi, saprophytes and parasites in general) derive food from other organisms and that the material oxidized by germinating seeds or by seedlings grown in darkness was not synthesized from water and carbon dioxide by themselves; in a very true sense such organisms are saprophytic. Also, if one cares to delve at all deeply, it is desirable to consider tissues and cells, and most tissues and cells, even of ordinary green plants, derive their food from other parts of the plant body.

The availability of an extensive series of quantitative observational data on carbon dioxide production and shoot elongation in very young "Nittany" wheat seedlings led me to try to compute approximately what portion of the water used in tissue enlargement might possibly have been formed within the plantlets and what portion might have been absorbed from without. The data referred to are those reported by Mack (1930) and by Mack and Livingston (1933), who have described the experimental technique employed and the averaging computations used. They are also the ones studied by Livingston and Mack (1934). (It is to be noted that Mack and Livingston consider values for the last 36 hours of the experiment period, while Livingston and Mack consider them for the whole period, and as relative values only; but data for computing the actual ones are given by the last-mentioned writers). The standard seedlings used had just protruded their coleoptiles. They were grown for 46 hours, submerged under dilute mineral nutrient solution; consequently their external supply of water was surely adequate at all times. They were in darkness, consequently there was no photosynthesis. The amount of carbon dioxide produced by each culture, of 100 seedlings, was ascertained and the average length of the ten longest shoots of each culture at the end of the experiment period was taken as an index of shoot elongation. A specified gas mixture continually bubbled through the solution in each culture flask and a large number of different proportions of oxygen and nitrogen were tested, both without and with ethylene (0.1 per cent. by volume). Each gas mixture was tested at each of five maintained temperatures (10°, 15°, 20°, 25°, 30°) and oxygen pressures of 0.6, 6.3, 9.8, 16, 20, 30, 50 and 75 per cent, by volume, were employed with ethylene, while the same oxygen pressures and also those of 90, 95 and 98.3 per cent were tested without ethylene. The present paper is concerned only with the data for the entire experiment period of 46 hours. The accompanying table presents the data for six different combinations of temperature and oxygen pressure, without ethylene, selected as representative of the whole range. For each combination the observational values are the weight of carbon dioxide produced

(C) and the average length of the ten longest seedlings (G). Results from several like tests are averaged in each instance.

The regular experimentation did not include measurements of all shoots nor did it include any root measurements at all. Some tests subsequently made by Mr. W. Luther Norem, of this Laboratory, showed that "Nittany" wheat seedlings very similar to the largest ones produced in the regular experimentation had generally a shoot diameter of about 1.2 mm., while they generally bore three roots each, about 0.6 mm. in diameter, each root being about 1.4 times as long as the shoot. For a very rough estimate, it may therefore be supposed that the increment of shoot volume per millimeter of length was generally about 2.3 cu. mm. and that the increment of root volume per millimeter of *shoot* length was generally nearly the same, about 2.4 cu. mm.; consequently the total volume increment of shoot and roots combined may be taken as about 4.7 cu. mm. per millimeter of shoot length. However, G is obviously larger than the average shoot length that might have resulted if all the shoots in each culture had been measured, but this inadequacy of the regular data may be approximately corrected by making the assumption that the average shoot length per culture was probably about half of the corresponding average for the ten longest shoots. To obtain an estimate of the total shoot-root volume increment per 100-seedling culture, this derived value ($G/2$) may be multiplied by 100, and $4.7 \times 100\ G/2$ may be taken as the estimated total volume increment per culture; that is 235 G, in terms of cubic millimeters.

The total shoot-root volume increment for a culture may safely be considered as very nearly equal to the volume of water incorporated in shoots and roots during the growth period of 46 hours; other materials than water may be regarded as playing a negligible part in the determination of the volume of such young tissues. Most of the water thus incorporated was doubtless derived by absorption from the surrounding nutrient solution but some portion of it must have been derived from respiration. To get a rough idea of the amount of respiration water produced by a culture that produced C mg. or carbon dioxide we may suppose that endosperm starch was the ultimate source of the material oxidized in respiration and that the chemical unit $C_6H_{10}O_5$ was completely oxidized in the process. From such oxidation the weight of water produced is about one-third (0.341) of the weight of carbon dioxide produced in the same time; for each 6 mols of carbon dioxide produced there should be produced also 5 mols of water, or 90 weight units of water for each 264 weight units of carbon dioxide. If these assumptions are regarded as legitimate, a culture with a total shoot-root volume increment of 235 G should have produced about $C/3$ mg. of water while giving off C mg. of carbon dioxide. The ratio of

the amount of water derived from respiration to the total amount incorporated in shoot and roots would thus be $C/3\times1/235\ G$, or $C/705\ G$, and this may be expressed as a percentage if multiplied by 100, giving 0.14 C/G. But R, the index of growth efficiency with respect to carbon loss (Livingston and Mack, 1934), is G/C and so the last expression may be written $0.142\times1/R$. Respiration water would thus supply more of the water incorporated by growth when R is small and less when R is large. Under conditions permitting much growth per unit of carbon dioxide produced, water of respiration would therefore be less important than when much carbon dioxide is produced per unit of growth. When metabolism is, as it were, wasteful of carbon, a considerable part of the growth water may be derived from respiration, but when there is relatively little waste of carbon the quantitative importance of respiration water is small.

Liaskovskii's analysis showed his cucurbit seeds to contain much fat and protein but only a negligible proportion of carbohydrate. According to his experiments, one weight unit of H_2O was producted for each 2–5 weight units of CO_2, the ratio of H_2O to CO_2 ranging from 1:2 to 1:4.9. In the present discussion this ratio is taken as 1:3, on the basis of the supposition that $C_6H_{10}O_5$ may fairly represent the material oxidized. If oxidation were incomplete, water production should of course be relatively less, and if material completely oxidized were such that its atomic ratio of H to C were greater than 10:6, water production should be relatively greater. According to Knüttel, Oudemans and Rauwenhoff's early experimental results lead to values of the ratio CO_2:H_2O that differ according to the plant form studied and according to the developmental stage of the seedlings considered, being apparently about 1·2 for their earliest germination stage of *Fagopyrum* and of *Brassica* but only about 1:50 for their earliest stage of *Pisum*. Knüttel quoted the Dutch writers as saying that water production was first less than carbon dioxide production but that the former increased more rapidly than the latter and surpassed it as development proceeded. For a later state of *Fagopyrum* their ratio is about 1:1/2; for a later stage of *Pisum*, about 1:2/3.

For each of six representative temperature-oxygen combinations, the accompanying table shows the primary values of G and C, also the estimated total amount of water incorporated in growing shoot and roots ($235\ G$) and the estimated amount of water produced by respiration ($C/3$). The latter is expressed in terms of milligrams for 100 seedlings in the 46-hour period and also as a percentage of the estimated total amount of water incorporated by growth. Finally, the value of the growth-efficiency index (R) is shown for the sake of comparison.

The combination of 30° with oxygen pressure of 90 per cent gave the

most rapid carbon dioxide production observed, with a growth-efficiency ratio (R) of 0.0172, which is relatively very low. For this combination it is estimated (see the table) that 940 mg. of water was incorporated in shoot and roots while 232.6 mg. of carbon dioxide was produced, along with 77.5 mg. of respiration water. Under these conditions the seedlings were obviously very wasteful of carbon but my method of approximation indicates that only about 91.6 per cent of the water incorporated by growth was absorbed from without, 8.2 per cent of it being derived from respiration. The combination of 30° with 20 per cent gave a similar low value of R (0.0169), showing a similar percentage importance of respiration water (8.4) but G is here only about half as great (2.2) as in the first case, C (130.2) being also much smaller.

Computation of respiration water for six representative combinations of temperature and O_2 pressure

Temperature—O_2 combination		30°, 20%	30°, 90%	25°, 75%	25°, 95%	20°, 20%	20°, 30%
Average length, 10 longest shoots, mm. (G)		2.2	4.0	8.0	12.2	7.2	9.2
Estimated mg. of H_2O incorporated by growth of 100 seedlings (235 G)		517	940	1880	2867	1692	2162
Mg. of CO_2 produced by 100 seedlings (C)		130.2	232.6	157.3	190.8	57.1	63.0
Estimated respiration water formed by 100 seedlings	Mg. ($C/3$)	43.4	77.5	52.4	63.6	19.0	21.0
	Percentage of incorporated H_2O (0.142 C/G)	8.4	8.2	2.8	2.2	1.1	1.0
Growth-efficiency index (C/G, or R)		0.0169	0.0172	0.0509	0.0639	0.1261	0.1460

For the combination of 25° with 75 per cent, only 2.8 per cent of the incorporated water appears to have been derived from respiration. This percentage is still smaller (2.2) for the combination of 25° with 95 per cent, which gave the greatest observed value of G. For the combinations 20° with 20 per cent. and 20° with 30 per cent, this percentage is very small (1.1, 1.0), the latter being the combination that gave the highest observed

value of R and the former being a common temperature-oxygen combination in nature and in greenhouses.

As was to be expected, none of the cultures showed respiration water as very important when compared with water derived from the surroundings, but nevertheless 8 per cent is quite considerable. On the basis of my assumptions and computations, it appears that high growth efficiency with respect to carbon loss (G/C) naturally goes with small importance of respiration water, and conversely. The relatively great importance of respiration water shown for the combinations 30° with 20 per cent, and 30° with 90 per cent occur for a temperature that is surely supra-optimal for general health of these seedlings, with relatively rapid carbon dioxide production (C), slow growth (G) and very low growth efficiency (R); perhaps all of the seedlings were somewhat unhealthy at 30°.

Of course this little discussion of the water relations of these very young seedlings is not to be considered as well grounded on quantitative observation, but respiration water is surely worthy of more theoretical and experimental attention than it has generally thus far received in connection with the physiology of the plant organism as a whole, and it appears that its quantitative importance may vary widely according to such environmental influences as mantained temperature and oxygen pressure. Direct measurement of rates of production of respiration water appears to be difficult in the present stage of physiological technique, but measurement may be made of rates of volume increase, dry-weight change, carbon dioxide production and oxygen absorption.

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INDEX TO AMERICAN BOTANICAL LITERATURE
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